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ECOLOGICAL DISTRIBUTION OF MAMMALS IN THE TULAROSA BASIN, NEW MEXICO

BY W. FRANK BLAIR

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INTRODUCTION

THE Tularosa Basin of southern New Mexico is an ideal situation for studying the relationships of mammals to their environment. This desert, virtually isolated on three sides by mountains or hills, is an area of a few hundred square miles containing extensive nearly white gypsum dunes and a large bed of dark lava, in addition to the characteristic ecological associations of the Chihuahuan Desert.

The unique characters of the Tularosa Basin have drawn the attention of several plant and animal ecologists. Ruthven (1907) studied the ecological distribution of the reptiles and amphibians of the basin and named most of the important ecological associations. He was the first to point out, in the lizards, the tendency of some species of animals of the White Sands to be lighter in color than those in near-by desert regions. MacDougal (1908) reported on the general plant ecology of the White Sands. Dice

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(1930) made a preliminary survey of the ecological distribution of the mammals of the basin and adjacent mountains. His discovery of an endemic, pale race of mammals on the White Sands and two endemic dark races on the lava bed (Dice, 1929) stimulated further work in these interesting areas. Bradt (1932) studied the mammals of the lava bed, Benson (1932, 1933) made an excellent and extensive study of the color of the mammals of the White Sands and dark lava bed, and Emerson (1935) made a reconnaissance study of the plant ecology of the White Sands.

I spent October, November, and a part of December, 1938, trapping breeding stocks of *Peromyscus* in the Tularosa Basin. Extensive trapping in several ecological associations resulted in an increase in the number of species of mammals known to inhabit several of the associations and added to our knowledge of the relative abundance of the several species. In March, April, and May, 1940, an intensive study of small mammal populations in the mesquite association was made, and a short time was spent investigating other associations. An annotated list of the mammals of the Tularosa Basin (Blair, 1941*a*) gives the locality data on which the present report is based.

These intensive investigations have resulted in the accumulation of more knowledge about the ecological distribution of the mammals of the Tularosa Basin than is available for any other desert area in North America. Much remains to be done in the study of the Tularosa Basin, particularly on the White Sands. Nevertheless, the data now available afford a rather comprehensive picture of mammalian distribution in the Tularosa Basin.

Acknowledgments

My field work in 1938 was supported by a grant from the Carnegie Institution of Washington to Lee R. Dice, Director of the Laboratory of Vertebrate Biology. The work in 1940 was made possible by a grant from the Horace H. Rackham School of Graduate Studies of the University of Michigan.

Tom Charles, Johnwill Faris, and George Sholly, of the National Park Service, rendered many courtesies while I was working at the White Sands National Monument. Ranchers Charles Bonnell, Gerald Champion, and Sam McNatt kindly gave me permission to trap on their ranches. I am obliged to C. L. Lundell, of the University of Michigan Herbarium, for identifying a grass that was collected in 1938. The plants that were collected in 1940 were kindly identified by A. L. Hershey, of the New Mexico State College. My wife, Fern Ann Blair, accompanied me on the trips and acted as field assistant.

ECOLOGICAL ASSOCIATIONS

An ecological association, as here defined, includes all of the plants and animals occurring together in a relatively stable environment. The mammals are, however, the only animal constituents treated in the present report.

There are four ecological associations on the desert plain of the Tularosa Basin. The creosote bush, mesquite, and atriplex associations occupy broad belts lying parallel to the mountain front and are encountered in that order as one proceeds from the base of the mountains to the center of the basin. The grassy wash association cuts across the other associations of the desert plain. The malpais lava bed has been described as one ecological association. Four associations are recognized in the White Sands. Several isolated, rocky buttes in the Tularosa Basin are not considered here, because no study has been made of their ecological associations.

In the treatment of individual associations an estimate of the average abundance of each species of mammal, based on my records and those of previous investigators, is given by means of a numerical scale containing five classes. These classes are: I (rare), II (few), III (intermediate in abundance), IV (common), and V (abundant). Classification of a species in a given association in one of these groups indicates its relative abundance as compared to its abundance and the abundance of other species in the other associations of the Tularosa Basin.

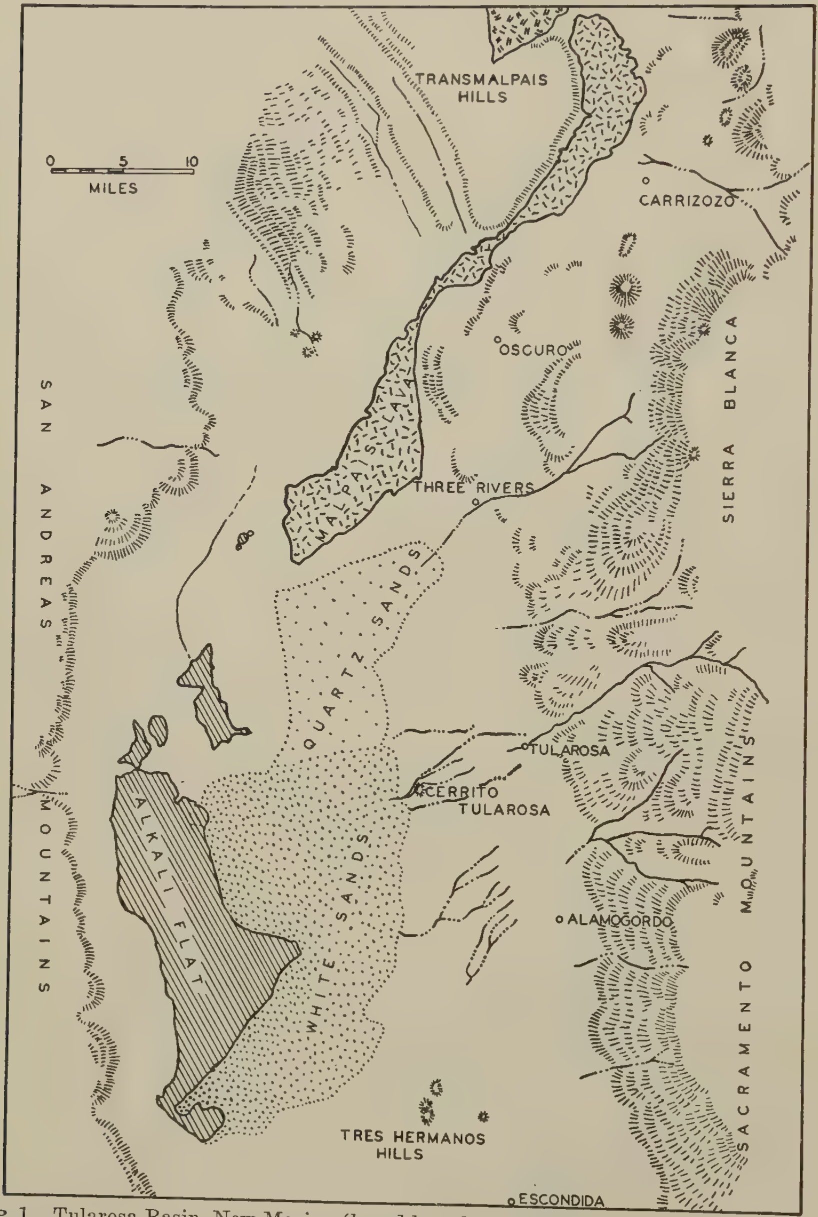
The estimated order of relative abundance of the mammals in each ecological association is given in the associational list of species. The species that is believed to be the most abundant one in the association heads the list. The other species follow in the probable order of their relative abundance.

The taxonomic arrangement of the mammals has been treated in a previous report (Blair, 1941*a*). Nomenclature of the plants, except grasses, is based on Wooton and Standley (1915), except for known changes. For the grasses, Hitchcock (1935) is used as an authority.

Creosote Bush Association

Mammals	Abundance
Perognathus penicillatus eremicus	V
Dipodomys merriami ambiguus	IV
Lepus californicus texianus	III
Sylvilagus audubonii minor	II
Onychomys torridus torridus	II
Neotoma micropus canescens	II
Thomomys baileyi tularosae	II (local)
Peromyscus maniculatus blandus	I
Dipodomys ordii ordii	I
Citellus variegatus grammurus	I
Peromyscus truei truei	I
Canis latrans subsp.	I

The creosote bush association was named and figured by Ruthven (1907), who studied it on the "alluvial slopes east of Alamogordo." Dice (1930) collected mammals in it near Alamogordo (Map 1). I trapped in this association about three miles north of Tularosa from October 8 to 12, 1938 (approximately 2000 trap nights). Pocket gophers were trapped in this association about two miles north of Alamogordo on February 29, 1940.



MAP 1. Tularosa Basin, New Mexico (based largely on Meinzer and Hare, 1915, Pl. 1).

The creosote bush association occupies the alluvial slopes along the base of the Sacramento Mountains in the Tularosa Basin. Therefore, there is a more or less continuous belt of this association, often several miles wide, which is bounded on the one side by the associations of the lower mountain slopes and on the other by the mesquite association. The creosote bush (*Larrea tridentata*) characterizes this association, and so far outnumbers other species of plants that they are scarcely noticeable. Herbs are, for the most part, conspicuously absent. Dice mentioned a small mustard as numerous in early July. The creosote bushes are generally separated by barren soil. Scattered mesquites (*Prosopis glandulosa*) occur in the association. In a few places creosote bush and mesquite are present in approximately equal abundance, but, usually, the creosote bush and mesquite associations are sharply defined.

The soil of the creosote bush association is highly variable in texture and color. Coarse sand predominates in many places, and in general the soil particles are coarser than they are in the mesquite association. Near the upper limits of the association, small rock fragments in some places litter the surface of the soil. The soil varies from dark red to light gray.

Two of the species of mammals recorded from the creosote bush association, *Citellus variegatus* and *Peromyscus truei,* are mostly limited to the rocky associations of the near-by mountain slopes and occur only rarely (class I) in the creosote bush association. All other mammals recorded from this association are of species that range widely through the desert associations of the Tularosa Basin. One species, *Thomomys baileyi,* is present in scattered, local colonies in the creosote bush, mesquite, and atriplex associations. The others, with the exception of *Perognathus penicillatus* and *Dipodomys merriami,* were notably scarcer in the creosote bush association in 1938 than they were in the mesquite association. The two exceptions to this rule (*Perognathus,* class V, and *Dipodomys,* class IV) made up the greatest bulk of the population of the creosote bush association in 1938.

Three species, *Neotoma micropus, Peromyscus maniculatus,* and *Dipodomys ordii,* were remarkably scarcer here than in the mesquite association. The difference in mammalian abundance in the two associations possibly is due in part to differences in the amount of available food. The mesquite plant, in some form or another, affords a food supply throughout the year. The creosote bush apparently furnishes food for the small mammals only in the form of seeds. Except when the autumn crop of creosote bush seeds is available, food for small rodents must be scarce in this association.

Mesquite Association

Mammals	Abundance
Dipodomys merriami ambiguus	V
Dipodomys ordii ordii	V
Perognathus penicillatus eremicus	V
Peromyscus maniculatus, blandus	V
Neotoma micropus canescens	IV
Onychomys torridus torridus	III
Citellus spilosoma major	III

Mammals	Abundance
Sylvilagus audubonii minor	III
Lepus californicus texianus	III
Peromyscus leucopus tornillo	III
Onychomys leucogaster ruidosae	II
Thomomys baileyi tularosae	II (local)
Reithrodontomys megalotis megalotis	II (local)
Peromyscus eremicus eremicus	II
Sigmodon hispidus berlandieri	I
Perognathus flavus flavus	I
Neotoma albigula albigula	I
Mus musculus	I
Cynomys ludovicianus	I (local)
Citellus variegatus grammurus	I
Canis latrans subsp.	I
Odocoileus sp.	I
Lynx sp.	I

Ruthven (1907) first named and described the mesquite association in the vicinity of Alamogordo. Dice (1930) studied its mammals near Alamogordo, and Benson (1933) collected mammals in an area of mesquite on red sand near Escondida. In 1938, I trapped mammals in this association about three to four miles south of Alamogordo from October 11 to 20 and from October 23 to 30, a total of approximately 7000 trap nights. An area of mesquite association on quartz sand about thirteen miles west of Tularosa was trapped on October 3 and from November 5 to 15, a total of approximately 4000 trap nights. An intensive study was made of the small mammal population on selected plots in the mesquite association about three miles south of Alamogordo from March 4 to May 24, 1940. These studies have provided more information about the mammals than is available for the mammals of any other association of the region, not excluding the more spectacular malpais lava and White Sands associations.

The mesquite association is markedly different at each of the three places it has been studied: near Alamogordo, where Dice and I worked; on the quartz sands, where I trapped; and on the red sands near Escondida, where Benson collected.

Near Alamogordo, the mesquite achieves a more luxuriant growth than anywhere else in the Tularosa Basin. Over an area of several square miles, lying in a strip between the creosote bush association and the atriplex association, the mesquite is the most abundant and the most conspicuous plant. The mesquite so dominates this association that all of the other plants could be removed without altering materially the appearance of the vegetation. The habit of growth of the mesquite varies greatly in this area. Some mesquites constitute small trees ten to fifteen feet in height, others form dense shrubby masses of varying heights, and yet others comprise extensive areas of closely spaced, short, relatively straight mesquite stems, usually less than two feet in height. The spacing of the mesquite bushes and clumps of bushes is highly variable. In some places more or less open thickets are formed. In most sections, however, extensive areas of barren soil intervene between the mesquites. A few scattered shrubs of other species, notably crucifixion thorn (*Koeberlinia spinosa*) and atriplex (*Atriplex canescens*) are present. Herbs are scarce. In April and May of 1940, *Lepidium alyssoides* was the most abundant herb. Four others, *Astragalus nuttallianus, Verbena wrightii, Gilia pumila,* and *Sophia* species, were scarce and local. The soil is relatively fine and predominantly grayish. Wind-blown sand and dust have accumulated around many of the mesquite clumps and built up mounds from a few inches to two or three feet high. Some of the mesquite clumps are without such mounds.

The area of mesquite association in the quartz sand region about thirteen miles west of Tularosa is very limited in extent. It covers at most one or two square miles. The mesquite clumps are much smaller and generally more scattered than near Alamogordo. *Atriplex* also occurs here. The soil is very fine and considerably gypseous. The surface of the soil is thinly crusted, due to the presence of gypsum, and is light gray. There is a slight accumulation of black alkali in some places.

The mesquite area trapped by Benson near Escondida is part of an extensive area of mesquite that extends south to Texas and nearly across the central Tularosa Basin on well-developed dunes of light red or orange sand. This area comprises several hundred square miles and is the largest unit of mesquite association in the basin. The mesquites grow extensively over the tops of the dunes. Grass and other herbs are more abundant than in the other areas of mesquite in the Tularosa Basin. No study of the vegetation of these dunes has been made. Benson reported that *Gilia longiflora* was an important food item in October and the only common annual in bloom at that time. The sand dunes of this association and the greater abundance of herbs distinguish it from the mesquite association near Alamogordo and west of Tularosa. Possibly it should be recognized as a distinct association, but until a more detailed study can be made it is convenient to include it in the mesquite association.

Twenty-two species of mammals have been recorded from the mesquite association, and one other species is reported to occur there. In this association, as represented at Alamogordo, five species account for the bulk of the mammalian population. These are *Dipodomys merriami, Dipodomys ordii, Perognathus penicillatus, Peromyscus maniculatus* (all class V), and *Neotoma micropus* (class IV). Five species (from *Onychomys torridus* through *Peromyscus leucopus* in the list at the beginning of the section) are considerably less abundant (class III) in the mesquite association than the above mentioned ones. All but one of these are widely distributed in the ecological associations of the basin, and are not numerous in any of them. The fifth species, *Peromyscus leucopus,* is mostly a constituent of the grassy wash association, where it ranks in class V.

Thirteen species of mammals are rare or local in occurrence in the mesquite association. Of these, *Thomomys baileyi* is present in colonies of considerable size, but the colonies are scarce. Another, *Reithrodontomys megalotis,* apparently is unevenly distributed or sporadic in the mesquite association, for, although Dice recorded thirty specimens taken in 1927, I caught none in two seasons of extensive work. This species becomes abundant (class V) in the grassy wash association. One species, *Sigmodon hispidus,* is largely restricted to the grassy wash association, where it is common (class IV). Two rare species of the mesquite association, *Perognathus flavus* and *Cynomys ludovicianus,* are relatively rare (class I or II) in all of the associations of the basin. The former is more abundant in the grassy wash (class II) than in the mesquite association (class I), and the latter has been partly destroyed by poisoning operations.

Three species that occur rarely or sporadically in the mesquite association are largely restricted to the rocky associations of the mountain slopes. One of the three, *Peromyscus eremicus,* was taken in the mesquite association in the fall of 1938, but it was not found there in the spring of 1940. Another, *Neotoma albigula,* was trapped there in 1940, but not in 1938. Dice found the third species, *Citellus variegatus,* to be scarce in the mesquite association in 1927, and I did not observe it there. Tracks of *Odocoileus* species were observed once in the mesquite association in the spring of 1940. The last species in the mesquite association list (*Lynx* species) was recorded by Dice as being reported to occur in the association.

The mesquite association about thirteen miles west of Tularosa was occupied by the species of mammals that were commonest in the same association near Alamogordo. Small mammals, however, were scarcer than they were in the Alamogordo mesquite. Three species, *Dipodomys merriami, Dipodomys ordii,* and *Peromyscus maniculatus,* made up the bulk of the small mammal population. Two species, *Perognathus penicillatus* and *Neotoma micropus,* were much scarcer at this station than at Alamogordo. *Lepus californicus* appeared to be about as plentiful as at Alamogordo. *Peromyscus leucopus* was scarce, for only one individual was caught. Only *Peromyscus eremicus* of the four characteristic mountain slope species recorded from the same association at Alamogordo was taken at this station. Although fewer species were recorded at this station than at Alamogordo, more extended trapping probably would reveal the presence of other rare species in this association. The sparse distribution and small size of the mesquite clumps probably accounts, in part at least, for the small mammals being scarcer here than in the Alamogordo mesquite.

The results of Benson's trapping in the mesquite association on the Escondida red sands point to marked differences from the other areas of mesquite association in the proportions of the several species recorded there. *Dipodomys ordii* apparently was the most abundant small mammal, followed rather closely by *Onychomys leucogaster,* which was rare at the other stations. *Sylvilagus audubonii* and *Lepus californicus* were recorded as numerous. *Dipodomys merriami* apparently was much scarcer than at the other stations in the mesquite association. *Peromyscus leucopus* was taken, but *Peromyscus maniculatus* was not. The striking differences in numbers between the sand dune mesquite association population and the populations of the same association on relatively level compact soils probably are attributable largely to these soil differences. The two most abundant species at the Escondida station usually are associated with sandy soil, although both occur away from sand.

Atriplex Association

Mammals	Abundance
Dipodomys merriami ambiguus	III
Dipodomys ordii ordii	II
Peromyscus maniculatus blandus	I
Citellus spilosoma major	I
Lepus californicus texianus	I
Thomomys baileyi tularosae	I (local)
Sylvilagus audubonii minor	I
Dipodomys spectabilis baileyi	I

Ruthven (1907) named and figured the atriplex association from his studies in the area between Alamogordo and the White Sands. Dice (1930) collected mammals in this association about nine miles west of Alamogordo. His is the only study of the mammals of this extensive association. In the spring of 1940, I trapped pocket gophers in this association about eleven miles southwest of Alamogordo.

The atriplex association occupies an extensive area in the Tularosa Basin between the mesquite association and the White Sands. The saltbush (*Atriplex canescens*) is the most abundant and most conspicuous shrub. Other plants recorded by Dice from this association are crucifixion thorn, prickly pear (*Opuntia* species), and bunch grass (*Sporobolus* species). I collected five species of plants in addition to saltbush about twelve miles southwest of Alamogordo: the joint fir (*Ephedra torreyana*), peppergrass (*Lepidium alyssoides*), blue curls (*Phacelia corrugata*), sand verbena (*Abronia angustifolia*), and a mallow (*Sphaeralcea* species). All of these, however, appeared to be relatively scarce and local in the atriplex association. This association occurs on predominantly flat land. The soil is highly alkaline and is mostly light gray.

All of the mammals recorded from the atriplex association, with the exception of *Dipodomys spectabilis,* are characteristic species of the mesquite association. They are, however, much scarcer in the atriplex than in the mesquite association.

Grassy Wash Association

Mammals	Abundance
Peromyscus leucopus tornillo	V
Reithrodontomys megalotis megalotis	V
Peromyscus maniculatus blandus	V
Sigmodon hispidus berlandieri	IV
Sylvilagus audubonii minor	III
Perognathus flavus flavus	II
Dipodomys merriami ambiguus	II
Dipodomys ordii ordii	II
Lepus californicus texianus	I
Neotoma micropus canescens	I
Peromyscus eremicus eremicus	I
Perognathus penicillatus eremicus	I
Neotoma albigula albigula	I
Peromyscus nasutus nasutus	I
Peromyscus truei truei	I
Onychomys leucogaster ruidosae	I
Notiosorex crawfordi crawfordi	I

Desert washes and ditches that transect the creosote bush, mesquite, and atriplex associations of the Tularosa Basin generally have dense growths of coarse grasses and numerous herbs. There also are scattered plants characteristic of the adjacent mesquite and creosote bush associations. Irrigation ditches and drainage ditches along railways have similar vegetation and are included in this association. The association was trapped extensively in the fall of 1938 from a point about three miles north of Tularosa nearly to Three Rivers, from November 22 to December 1 and from December 3 to 9, a total of approximately 6000 trap nights. In March, 1940, an area about three miles southwest of Alamogordo was trapped for several nights. The alkali meadow community described by Dice (1930) apparently should be included in this association.

The plants of this association have not been carefully studied. During both of my visits to the basin the herbs were not in condition to be identified. Coarse bunch grasses are the most important feature. Near Tularosa the Mexican gourd (*Cucurbita foetidissima*) is an important plant. The vines cover extensive areas, and the seeds of this gourd are an important source of food for small mammals. A few mesquites, creosote bushes, and saltbushes occur. In the area he studied Dice mentioned "a fairly thick stand of tall grasses, some herbs, and a few scattered low shrubs."

Seventeen species of mammals have been recorded from the grassy wash association. Four species, *Peromyscus leucopus, Reithrodontomys megalotis, Peromyscus maniculatus* (all class V), and *Sigmodon hispidus* (class IV), comprise the bulk of the population. It is particularly interesting that here small mammals of the family Cricetidae are the most abundant species, while in the other associations of the basin mammals of the family Heteromyidae tend to predominate in numbers. The cottontail (*Sylvilagus audu-*

bonii), class III, and a pocket mouse (*Perognathus flavus*), class II, become more abundant here than in any other association of the basin.

Eleven species are relatively rare (class I and II) in the grassy wash association. Six of these, *Dipodomys merriami, Dipodomys ordii, Lepus californicus, Neotoma micropus, Perognathus penicillatus,* and *Onychomys leucogaster,* are characteristic inhabitants of the adjacent associations of the desert plain and probably range from those into this association. A shrew (*Notiosorex crawfordi*) is recorded in the basin only from this association.

Four of the species that are relatively rare in the grassy wash association, *Peromyscus eremicus, Neotoma albigula, Peromyscus nasutus,* and *Peromyscus truei,* are characteristic inhabitants of rocky, mountain slopes, where they usually are abundant.

Malpais Lava Association

Mammals	Abundance
Perognathus intermedius ater	IV
Peromyscus nasutus griseus	III
Peromyscus eremicus eremicus	III
Neotoma albigula melas	III
Citellus variegatus tularosae	II
Peromyscus truei truei	II
Pipistrellus hesperus maximus	II
Sylvilagus audubonii minor	I
Lepus californicus texianus	I
Neotoma mexicana atrata	I
Citellus interpres	I
Spilogale leucoparia	I
Eutamias cinereicollis canipes	I
Dipodomys spectabilis baileyi	I
Antrozous pallidus pallidus	I
Lynx rufus baileyi	I

Recorded from the Edge of the Lava

Perognathus flavus flavus
Dipodomys ordii ordii
Neotoma micropus canescens
Reithrodontomys megalotis megalotis
Peromyscus leucopus tornillo

The malpais lava association of the Tularosa Basin was named and figured by Dice (1930), who (1929) named two dark races of mammals from the malpais. The mammals were studied by Bradt (1932) and also by Benson (1933), who (1932) described two dark races of mammals from the malpais. I trapped this association about four miles west of Carrizozo on the nights of October 21 and 22, 1938, and about five miles west of Oscuro for a few nights in late April and early May, 1940.

The Tularosa malpais or lava bed lies in the northern part of the basin. The lava flow is more than forty miles long and varies in width from less than one mile to about six miles. Dice has given a general description of the malpais lava association near its southern end. He noted that, "there is a sparse growth of mesquite, several kinds of cacti, some unidentified shrubs, and a very little grass.'' Benson likewise gave a general description of the association and pointed out that the juniper (*Juniperus monosperma*) is the dominant plant at the northern end of the lava bed and that mesquite and creosote bush are the chief plants at the southern end. No adequate study of the plants of this association has been made. I noted that the vegetation seemed to be in a much more normal condition west of Oscuro than west of Carrizozo and at the southern end of the bed, where there was evidence of intensive grazing by goats and cattle. The vegetation on the lava bed west of Oscuro probably is representative of the natural vegetation of the malpais, except at its northern end, where junipers predominate.

West of Oscuro, the principal plants of the relatively level, more or less unbroken lava surfaces are snakeweed (*Gutierrezia* species), woolly groundsel (*Senecio longilobus*), purple dahlia (*Parosela formosa*), cholla cactus (*Opuntia* species), yucca (*Yucca* species), streptanthus (*Streptanthus valida*), saltbush (*Atriplex canescens*), rattail cactus (*Opuntia leptocaulis*), and a grass that could not be identified at the season of field work. On the level surfaces, these plants grow in small crevices in the lava, where slight quantities of soil have accumulated.

The plants of the level lava surfaces also grow on rough, broken lava, in lava sinks, or on cliffs, where an additional conspicuous plant is a sumac (*Rhus microphylla*). Red salvia (*Salvia henryi*) also grows in these places. A few mesquites grow on both the broken and relatively unbroken lava surfaces.

Other plants identified from the malpais lava association west of Oscuro include bitterweed (*Actinea mearnsii*), borage (*Cryptanthe crassisepala*), erigeron (*Erigeron* species), Apache plume (*Fallugia paradoxa*), blue curls (*Phacelia caerulea*), clammy weed (*Polanisia* species, probably *uniglandulosa*), bladderpod (*Lesquerella* species), tansy mustard (*Sophia halictorum*), and verbena (*Verbena* species, probably *wrightii*). This list of plants undoubtedly is far from complete, being representative of only one season of the year. It includes, however, the species that characterize the association throughout most of the year and indicates that the malpais lava association probably supports a greater variety of plant life than any other association of the Tularosa Basin.

The physical features of the lava bed already have been adequately described by Meinzer and Hare (1915), by Dice (1930), and by Benson (1933). Perhaps emphasis should be placed on the probable importance of lava sinks, as represented west of Oscuro. Some of these are twenty to thirty feet deep and lead into small caves beneath the lava. The temperature in these sinks and caves is much more even than on the surface of the bed. Many of the deep, well-protected sinks and caves are moist at the bottom, and such mesic animals as craneflies, which probably could not exist on the surface of the lava, were observed in some of them. On the hottest days, when the surface of the lava was nearly unbearable because of the absorption of heat by the dark rock, the sinks were pleasantly cool.

Twenty-one species of mammals have been recorded from the malpais lava association, but five of them, *Perognathus flavus, Dipodomys ordii, Neotoma micropus, Reithrodontomys megalotis,* and *Peromyscus leucopus,* apparently range only into the edge. The five species that have been recorded only from the edge of the lava bed are characteristic inhabitants of other associations of the Tularosa Basin.

Five species, *Perognathus intermedius* (class IV), *Peromyscus nasutus, Peromyscus eremicus,* and *Neotoma albigula* (all class III), and *Citellus variegatus* (class II), are the most abundant forms in the malpais lava association and apparently range over the entire lava bed. Endemic, dark races of all of these except *Peromyscus eremicus* have been described and named. The population of *Peromyscus eremicus* on the malpais lava is highly variable, as Benson (1933) already has pointed out. Some individuals are dark; others are light.

The canyon bat (*Pipistrellus hesperus*) was found by Dice to be abundant at the southern end of the lava bed, and it is to be expected that it ranges over the entire association. Ten species of mammals appear to be relatively rare in this association. A dark endemic race of one of these, *Neotoma mexicana,* has been described. The one specimen of another, *Eutamias cinereicollis,* collected by Benson (1933) was stated by him to be dark.

White Sands Associations

Mammals	Abundance
Range Over the Entire Area	
Geomys arenarius brevirostris	IV
Perognathus apache gypsi	III
Peromyscus maniculatus blandus	III
Citellus spilosoma major	III
Dipodomys ordii ordii	II
Dipodomys merriami ambiguus	II
Perognathus flavus flavus	I
Lepus californicus texianus	I
Neotoma micropus leucophaea	I
Canis latrans subspecies	I
Vulpes macrotis neomexicana	I
Antilocapra americana subspecies	I
Erethizon epixanthum subspecies	I
Odocoilus species	I

Recorded Only from the Edge

Onychomys leucogaster ruidosae
Perognathus penicillatus eremicus
Onychomys torridus torridus
Reithrodontomys megalotis megalotis
Sylvilagus audubonii minor
Cratogeomys castanops lacrimalis
Taxidea taxus berlandieri
Dipodomys spectabilis baileyi
Cynomys ludovicianus arizonensis

The White Sands area was figured by Ruthven (1907), who named one ecological association, the sumac-yucca association. Dice (1930) studied the mammals of the sumac-yucca association at the edge of the White Sands. He named another association, the grama grass-joint fir association, but did not study its mammals. I trapped in the interior of the White Sands from November 16 to 20, 1938 (approximately 2000 trap nights). This station is in section 19, township 17 south, range 7 east on the Point of Sands quadrangle. It is about four miles from the eastern edge of the White Sands and is representative of the interior of the sands. From observations at this station, it seems desirable to name two ecological associations in addition to the two that previously have been recognized in the White Sands. Both of these occupy the valleys between the dunes and will be designated, respectively, the wet valley and dry valley associations. In 1940 I trapped briefly in the interior and near the edge of the White Sands.

Benson (1933) made an extensive study of the mammals of the White Sands, but he was concerned primarily with color variation rather than ecological distribution. His work on the White Sands was limited to three stations on the eastern edge. MacDougal (1908: 11) gave a general description of the vegetation of the White Sands and a chemical analysis of the gypsum "sand." Emerson (1935) made a general survey of the plant ecology of the White Sands, but he did not investigate the distribution of plant associations in the area. As a matter of fact, no detailed study of the separate ecological associations of the White Sands has been made, although a considerable amount of work has been done on the entire area as a biotic unit. Consequently, it seems best for the present to characterize the recognizable ecological associations as adequately as possible and to discuss the mammalian fauna of the entire White Sands area as a unit.

The sumac-yucca association occurs on many of the gypsum dunes of the White Sands. There is, however, no vegetation on many of the active dunes, particularly in the interior of the area. On the other dunes, the density of the vegetation varies greatly. The plant cover is relatively heavy on the more or less stable dunes near the edge of the White Sands, where there are numerous annuals in addition to the seven herbaceous species that Emerson (1935: 232) found able to elongate and thus keep above the encroaching sand. These last, which include *Ephedra torreyana, Yucca elata, Populus wislizeni, Atriplex canescens, Rhus emoryi, Poliomintha incana,* and *Chrysothamnus latisquameus,* because of their peculiar adaptability, extend into the interior of the White Sands, where they are virtually the only plants of the sumac-yucca association. The vegetation usually is sparse on the interior dunes, in contrast with its relative thickness on the peripheral dunes.

The grama grass-joint-fir association occupies the valleys between the dunes near the edge of the White Sands. Most of the sixty-two species of plants recorded by Emerson (1935: 230) from the White Sands occur in this association, and most of them apparently are limited to it. There is an excellent ground cover of grama grass (probably mostly *Bouteloua breviseta,* recorded from the White Sands by Emerson, 1935). There are scattered, small thickets of sumac (*Rhus emoryi*). The gypsum is encrusted in these valleys.

The wet valley association occupies low valleys between the dunes in the interior of the White Sands, where the water table is near the surface. The surface of the gypsum soil is encrusted and usually moist. Water undoubtedly stands in these valleys at some times. The vegetation grows on small hummocks projecting above the general level of the valleys. The principal vegetation consists of a coarse bunch grass (possibly Indian rice grass, *Oryzopsis hymenioides,* which was recorded from the interior of the sands by Emerson) and joint fir (*Ephedra torreyana*).

The dry valley association occupies relatively dry valleys in the interior of the White Sands. The soil is not encrusted, but is made up of gypsum particles similar to those that comprise the dunes. The principal plant of this association is prairie beard grass (*Andropogon scoparius* var. *neomexicanus*). This, with yucca (*Yucca elata*), makes up the bulk of the vegetation.

Twenty-three species of mammals have been recorded from the White Sands, but only eleven of these are known to range into the interior of the sands. A pocket gopher (*Geomys arenarius*), class IV, probably is the most abundant mammal of the White Sands. The wet and dry valley associations of the interior are literally undermined by the tunnels of this mammal. It is abundant in the grama grass-joint fir association of the periphery, but is relatively rare in the sumac-yucca association. Three other species, *Perognathus apache, Peromyscus maniculatus,* and *Citellus spilosoma* (all class III), are listed in the order of probable abundance. All of these live in all of the ecological associations, but in the interior they are more numerous in the wet and dry valley associations than in the sumac-yucca association. At the periphery of the area they all probably exceed *Geomys* in abundance, but if the entire population of the White Sands is considered *Geomys* probably leads in numbers. One of these species, *Perognatus apache,* is represented on the White Sands by an endemic, pale race, *gypsi.* Another, *Citellus spilosoma,* is paler and has larger white dorsal spots than others of the same species living in the mesquite association near Alamogordo (Blair, 1941*b*). The pocket gopher, *Geomys arenarius,* is represented by the endemic race *brevirostris,* which is not notably pale.

Three species of mammals, *Dipodomys ordii* and *Dipodomys merriami* (class II) and *Perognathus flavus* (class I), are more numerous at the edge of the White Sands than they are in the interior, where they are mostly limited to the dry valley association. Two species of carnivores, *Canis* *latrans* and *Vulpes macrotis*, are present in the interior of the sands as well as at the edge. *Lepus californicus* and *Neotoma micropus* range into the interior, but they are rare there as well as at the periphery. The latter is represented in the White Sands by the endemic race *leucophaea* (Goldman, 1933), which is described as being paler than *canescens.*

Some of the nine species of mammals recorded from the edge of the White Sands possibly will be found to range into the interior. Three other species undoubtedly are rare or transient in the White Sands. Dice reported *Antilocapra americana* from the area. I am at a loss to explain the origin of remains of *Erethizon epixanthum* that were found in 1940. The deer tracks that Ranger George Sholly reported probably were made by an individual from the San Andres Mountains, which lie west of the White Sands.

ISOLATION AND DISPERSAL OF MAMMALS IN THE TULAROSA BASIN

Isolation and natural selection appear to be the most important factors involved in the formation of local races of southwestern mammals (see Dice and Blossom, 1937 : 116; and Benson, 1933 : 56). Ecological isolation in the Tularosa Basin tends to break some of the species of mammals into numerous relatively small, more or less isolated population units. In these units the evolution of local races through natural selection may be accelerated. In addition, differentiation may occur in small populations through random fixation of genes (see Dobzhansky, 1941 : 168).

The tendency for small, partly isolated populations to differentiate is inhibited by the interchange of individuals between the several populations. This interchange acts to maintain the uniformity of the entire population of the species (see Dobzhansky, 1941 : 334). Wright (1931 : 126) has devised a formula for measuring the effectiveness of migration (dispersal) in retarding differentiation in partly isolated populations. As he points out, the theoretical case on which the formula is built must be much qualified before it is applicable to the conditions that actually obtain in natural populations. He does make the general statement, however, that, "it would appear that an interchange of the order of thousands of individuals per generation between neighboring subgroups of a widely distributed species might well be insufficient to prevent a considerable random drifting apart in their genetic constitutions." It is to be noted that he is speaking here of random divergence. With selection in different directions taking place in each of two neighboring populations, differentiation could be accomplished with an even greater interchange of individuals. Dice and Blossom (1937 : 116) cite instances of differentiation occurring presumably through natural selection though there was no apparent physical barrier to interbreeding.

Of importance in ecological isolation in the Tularosa Basin is the fact that the mammals of the region, as represented by nine species that have been studied, have home ranges that are occupied over a relatively long period of time (Blair, in press). The existence of these home ranges, each comprising a few acres, helps to localize the individual mammals and thus retards the spread of hereditary characters through the entire population. Occasional changes of home range occur, and movements of as much as one-third of a mile sometimes are made. These movements accelerate the spread of inherited characters throughout the population. With these observations in mind the possible role of ecological isolation in the differentiation of local races in the Tularosa Basin will be considered.

Desert Associations as Barriers to Mammals of the Desert Plain

The grassy wash and mesquite associations of the desert plain both include a considerable number of species of small mammals. Several species are abundant in these associations. It is evident then that these associations provide favorable ecological conditions for these species. The creosote bush and atriplex associations, on the other hand, have relatively few species of mammals, and most of the species are scarce in these associations. This scarcity seems attributable to the limited supply of food and cover available. The creosote bush and atriplex associations, being ecologically unfavorable to most small mammals, act as more or less effective barriers to the interchange of individuals and genes whenever they intervene between areas of the other associations.

The grassy wash association, because it is limited to desert washes and ditches, is discontinuously distributed in the Tularosa Basin. Since the washes cut across the belts of creosote bush and mesquite associations, small areas of grassy wash association comprising only a few acres to a few hundred acres may be completely surrounded by one or both of these other associations. Neighboring areas of grassy wash association may be separated by from less than one mile to several miles of other associations. Near Tularosa the isolation from one another of successive desert washes has ceased to exist since the building of the Southern Pacific Railway through the basin. The drainage ditches along the right of way are occupied by grassy wash association plants and mammals, thus making one panmictic breeding unit of many small populations that previously were isolated by the creosote bush association.

Three of the four characteristic and most abundant species of the grassy wash association, *Peromyscus leucopus, Reithrodontomys megalotis,* and *Sigmodon hispidus,* never have been recorded from the creosote bush association. Extensive trapping in this association within a few hundred feet of the grassy wash association when population pressure was at a peak there failed to take a single individual of any of these species. All are closely associated with grass and other heavy ground cover in the southwestern desert regions, and *Sigmodon* is conspicuously adapted morphologically to such an environment. For these species, the creosote bush association, consequently, affords an effective barrier to dispersal.

One species, *Peromyscus maniculatus,* which is characteristic of the grassy wash association and abundant there, occurs but rarely in the creosote bush association. For this species, and for the three discussed above, there can be little doubt that the creosote bush association acts as a highly effective, although probably not complete, barrier to dispersal.

Where units of grassy wash association are surrounded by the mesquite association, the isolation of these units apparently is less effective than where they are surrounded by creosote bush association. For *Peromyscus maniculatus* the mesquite association is no barrier, because this species is one of the characteristic and abundant species there. For *Peromyscus leucopus* and *Sigmodon hispidus* this association is a partial barrier, which is most effective for the latter. *Sigmodon* was taken rarely in the mesquite association and only at a time when a peak population existed in the grassy wash association. *Peromyscus leucopus* was fairly common locally in the mesquite association when populations were high, and a few were found locally even when populations were not at a peak. *Reithrodontomys megalotis* has been recorded from the mesquite association only where there was an unusual variety and abundance of vegetation. The mesquite association, as represented at most places in the Tularosa Basin, apparently is an effective barrier to this species. In general, the evidence indicates that the mesquite association is a highly effective barrier to the dispersal of *Sigmodon* and that this association acts to retard the dispersal of *Peromyscus leucopus* and *Reithrodontomys megalotis.* Even in the case of *Peromyscus* and *Reithrodontomys* this barrier probably is fully adequate to permit random divergence in neighboring populations if Wright's statement (1931: 126) is taken as a rough criterion of the isolation necessary for this to take place.

The mesquite association, in the Tularosa Basin, occurs as rather widely separated units. One such unit is the area of several square miles of mesquite immediately south of Alamogordo. Another, comprising several hundred square miles, occurs on the Escondida red sands. A small unit of only one or two square miles is situated on the quartz sands west of Tularosa. There are other such units in the northern and western parts of the basin. These units are separated by wide expanses of atriplex association, creosote bush association, or both. *Peromyscus maniculatus* and *Neotoma micropus* of the mesquite association seem to find these intervening associations a fairly effective barrier to dispersal. For some of the other species these unfavorable associations probably also are partially effective as barriers, because the species are relatively scarce in them.

Isolation and Differentiation of Mammals on the White Sands

The White Sands comprise approximately 270 square miles of dazzling white gypsum dunes near the middle of the Tularosa Basin. They are surrounded by atriplex association and on the west by an alkali flat that presents even more adverse conditions as a mammalian habitat than does the atriplex association. The mammals of the White Sands are mostly those that occur in abundance in the mesquite association or that are widely distributed in most of the ecological associations of the desert plain. Several of these species, however, are known to occur rarely in the atriplex association, and some of them have not been recorded from that association. Consequently, the rather considerable populations of mammals living at the edge of the White Sands are, to some extent, isolated from populations of the same species living in the distant mesquite association. For example, the White Sands are separated from the Alamogordo mesquite by approximately ten miles of atriplex association. At least two of these species apparently are undergoing adaptive differentiation on the White Sands. A local race of the wood rat (*Neotoma micropus leucophaea*) has been described from the White Sands by Goldman (1933: 472). The describer stated that this is a "slightly differentiated" race, paler than *canescens* of other parts of the Tularosa Basin. The spotted ground squirrel (*Citellus spilosoma major*) is paler and has larger white spots on the White Sands than it does on the darker soil of the mesquite association near Alamogordo (Blair, 1941*b*). The Chihuahua deer-mouse (*Peromyscus maniculatus blandus*) also tends to be paler on the White Sands than in some other populations in the Tularosa Basin. This is shown by a detailed study of color variation of this mouse in the Tularosa Basin now in progress.

Two species of mammals of the White Sands never have been recorded from any other ecological associations of the Tularosa Basin. Significantly, both of these species are represented on the White Sands by endemic races. One of these, *Perognathus apache gypsi,* is extremely pale and, consequently, is well adapted for concealment on the White Sands. The other, *Geomys arenarius brevirostris,* while a well-marked race, is not adaptively colored. Actually, *brevirostris* is darker than another race living on darker soil in the Rio Grande Valley from El Paso to Las Cruces.

Benson (1933: 25) in attempting to explain this anomalous phenomenon stated that these pocket gophers tend to match the wet soil they push out of their burrows. He also said that the pocket gophers are most abundant in low places, where the soil is wet and consequently yellowish. The gypsum sand, when wet, is somewhat darker than when dry, giving an Ives tint photometer reading of 58 per cent for reflected red compared with a value of 70 per cent for the dry sand. The wet sand, however, lacks much of being as dark as the local race of pocket gopher. Two specimens of this animal from the White Sands give an average tint photometer reading of 18.5 per cent for red. A specimen of *Geomys arenarius arenarius* from Kenzin, Dona Ana County, has a tint photometer reading of 21 per cent for red. Both races are relatively pale and tend to match the pale desert soils of southern New Mexico. The fact remains, however, that *brevirostris* is much darker than either the dry or wet gypsum, and it is darker than another race living on darker soil than the gypsum.

The adaptively colored pocket mouse also is darker than either the wet or dry sand and gives a lower mean tint photometer value (see Dice and Blossom, 1937: 79). The difference, however, between the mean pelage color of the pocket mice and the mean color of the dry sand is considerably less than that between the pocket gopher and even the wet sand. The palest pocket mice approach the light color of the sand much more closely than do any of the pocket gophers. Actually, the tint photometer readings of the pocket mice do not show adequately the full extent of the adaptive coloring of these animals, for the readings are made on the dorsal stripe, which is the darkest part of the animal.

Benson's statement that the *Geomys* are exposed to attack when on the soil shoved out of their burrows, and his inference that selection is toward a pelage that matches this soil is scarcely defensible. In the interior of the White Sands the species is very abundant in the dry valley association, where the sand is dry and white. Trails in the sand indicate that the pocket gophers often leave their burrows and travel overland for several feet to gather food. Many of the burrows have these trails radiating out in several directions from them. It can hardly be doubted that the animals are more exposed to attack when several feet from their burrows than when they are at the entrances to them.

The endemic race of *Geomys* on the White Sands apparently has originated through random differentiation in an isolated population, for its principal characteristics are cranial and presumably nonadaptive. The failure of this pocket gopher to have developed a pelage in harmony with the light background suggests that colonization of the White Sands by *Geomys arenarius* has been a rather recent event. The relatively dark color of this race possibly was fixed before the species invaded the White Sands. The White Sands are on the extreme periphery of the range of the genus and species, and an extension of the geographic range may well have occurred in comparatively recent time. Pocket gophers, as a group, match rather closely the soil on which they occur. This being the case, it is logical to believe that *Geomys* has inhabited the White Sands too short a time to become adaptively colored. The Escondida red sands, which extend southward into Texas, afford a logical invasion route from the population of *Geomys arenarius* inhabiting the Rio Grande Valley. This species was trapped in a very similar sand area at Kenzin. If, as I believe, the Escondida sands have been the avenue by which *Geomys* reached the White Sands the species apparently later became extirpated there, for there are no records of *Geomys* from there.

One cannot but note that, in spite of its lack of protective coloration, *Geomys* is one of the most abundant species of mammals on the White Sands. Since pocket gophers spend much more time underground than do most small mammals they undoubtedly are less subject to attack by predators than are the other forms. This being the case, natural selection theoretically should operate more slowly in bringing the pelage of these subterranean mammals into harmony with their environment than it would operate on species that spend much of their time above ground. Nevertheless, in the course of future evolution, natural selection theoretically should produce a pale race of *Geomys* on the White Sands.

In general, species of mammals that appear to be completely isolated on the White Sands are represented there by well-marked endemic races. One of these races, *Perognathus apache gypsi,* is adaptively modified; the other, *Geomys arenarius brevirostris,* apparently represents random differentiation. Some of the species that are partly isolated from other populations in the basin by the unfavorable atriplex association exhibit a trend toward adaptive differentiation on the White Sands. No detailed comparisons between populations of most of the partly isolated species have been made.

Isolation and Differentiation of Mammals on the Malpais Lava

The striking examples of protectively colored mammals to be found on the malpais lava bed already have been discussed in detail (Bradt, 1932; Benson, 1933; and Dice and Blossom, 1937). Five endemic races, characterized by dark pelages that somewhat match the dark lava rock, have been described. In addition, *Peromyscus eremicus* is represented by a variable population in which a fairly high percentage of the animals are dark. The one specimen of *Eutamias cinereicollis* known from the association was stated by Benson (1933) to be dark. Thus, seven species have undergone or appear to be undergoing adaptive differentiation on the malpais lava.

Previous workers have attributed the development of the dark races on the malpais lava to the combined effects of isolation and natural selection, with which view I am in accord. It should be emphasized, however, that the isolation of these species on the malpais lava is far from complete. All of these species are abundant in the mountains that border the Tularosa Basin, and in one place, according to Bradt (1932), the malpais is in contact with the foothills of these mountains. The malpais is very close to these hills along much of its western edge. Furthermore, there are scattered hills only a few miles apart between the eastern edge of the malpais and the mountains. Four of the species that are differentiated on the malpais have been taken in desert associations of the basin. Individuals of these species, which include *Citellus variegatus, Peromyscus eremicus, Peromyscus nasutus,* and *Neotoma albigula,* easily could reach the malpais from the hills to the west or from the mountains to the east by way of the scattered hills and buttes. They could come from either direction by crossing two or three miles or less of desert between areas of favorable ecological conditions. All four of these species have been found in desert associations at least two or three miles from the mountains. It probably is significant, as Benson (1933) has pointed out, that *Peromyscus eremicus,* which is the most variable of the differentiated lava bed mammals, occurs in desert associations in greater numbers and farther from the mountains than do the other species. It seems likely that more individuals of *eremicus* than of the other species reach the malpais from near-by areas of light rock.

Briefly, the development on the malpais of dark races of species of which relatively light races occupy the near-by mountains indicates that natural selection is sufficiently rigorous there to produce adaptive differentiation. The evolution of these dark races has occurred and is occurring in spite of the fact that the malpais populations are only partly isolated. No measure of the interchange of individuals of any species between the malpais population and the populations of near-by hills has yet been made. The close proximity to hills along much of the malpais suggests that there probably is a sufficient interchange of individuals to prevent random divergence of the populations, if the general statement of Wright (1931: 126) is taken as a rough indication of the amount of interchange necessary. That differentiation has taken place and is taking place apparently is due to selection pressure sufficiently strong to neutralize and to overcome the effect of incoming genes.

Associations of the Desert Plain as Barriers to Mammals of the Mountains

The slopes of the mountains near the Tularosa Basin and isolated buttes in the basin are inhabited by several species of mammals that are more or less restricted to rocky situations. Others, while not rock inhabitants, are largely restricted to the ecological associations of the mountain slopes. The desert plain of the Tularosa Basin is, in part at least, a barrier to the dispersal of these species. That the Tularosa Desert is not a complete barrier to several of these mammals is indicated by the fact that seven of them have been recorded from the ecological associations of the desert, at least two or three miles from the nearest rocky situations at the base of the mountains. *Citellus variegatus* was observed in the creosote bush association, and Dice (1930) recorded it from the mesquite association. *Peromyscus truei* was trapped in the creosote bush and grassy wash associations. *Peromyscus eremicus* and *Neotoma albigula* were taken in the mesquite and grassy wash associations. *Peromyscus nasutus* was taken in the grassy wash association. Tracks of *Odocoileus* species were seen in the mesquite association and on the White Sands. Remains of *Erethizon epixanthum* were found on the White Sands. Of all these species, *Peromyscus eremicus* occurs on the desert in greater numbers than does any other of these species and has been recorded farthest from the mountains. Nine individuals of this species were trapped in mesquite association near the middle of the basin. At the time when most of these species of small mammals were trapped on the desert, the fall of 1938, populations were at a peak. Population pressure must then have been heavy in their normal habitats.

Although these several species of saxicolous mammals of the mountain slopes have been taken in the Tularosa Desert, it seems certain that isolation of scattered buttes in the center of the basin by the desert associations must be highly effective, except for *Peromyscus eremicus.* Benson (1933) recorded *Perognathus intermedius, Peromyscus eremicus,* and *Neotoma albigula,* from Cerrito Tularosa, an isolated, rocky peak southwest of Tularosa. There probably is some interchange of genes between the population of this peak and the populations of other similar, partly isolated peaks and buttes such as the Tres Hermanos group southwest of Alamogordo. There probably is also some transfer of characters between these desert butte populations and the populations of the mountains that front on the desert. This interchange, however, appears to be too slight to prevent theoretically random divergence in the partly isolated populations.

SUMMARY

Four major ecological associations occupy the desert plain in the Tularosa Basin. Four other ecological associations are recognized on the White Sands, and the malpais lava comprises still another association. In the Tularosa Basin certain species of mammals are largely restricted to the grassy wash or mesquite association, and they occur only rarely in the creosote bush and atriplex associations. The unfavorable creosote bush and atriplex associations, consequently, somewhat isolate the populations of neighboring, scattered areas of the grassy wash and mesquite associations. Thus, there are, in the Tularosa Basin, numerous relatively small populations of mammals that appear to be sufficiently isolated to permit theoretically random divergence in their genetic constitutions.

Several desert species of mammals appear to be developing adaptively modified races on the White Sands, where they are partly isolated by the atriplex association. Two species that apparently are completely isolated on the White Sands are well differentiated there. One of these is protectively colored; the other seems to represent random differentiation.

Seven species of mammals have undergone or appear to be undergoing adaptive differentiation on the malpais lava. These populations are but little isolated from the populations of near-by mountains and hills. Therefore, selection on the malpais lava appears to be sufficiently rigorous to overcome the nullifying effects of a considerable interchange of individuals between this and neighboring populations.

The Tularosa Desert serves to isolate populations of saxicolous mammals living on scattered buttes in the desert and in the mountains bordering it. That this isolation is not complete is indicated by the fact that seven species of mammals usually restricted to rocks and mountain slopes have been recorded from the desert.

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POPULATIONS OF THE DEER–MOUSE AND ASSOCIATED SMALL MAMMALS IN THE MESQUITE ASSOCIATION OF SOUTHERN NEW MEXICO

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INTRODUCTION

THE size of the home range and the rate of dispersal of a species must be learned before any attempt can be made to determine the rate at which local races of that species originate. Population size, rate of increase, and social relations within the species also are important because of their possible effects on the origin of local races.

As a part of an attempt to learn the rate of origin of local races of deer-mice in the southwestern United States, live trapping and marking operations were carried out in the mesquite association near Alamogordo, New Mexico. The trapping was begun on March 4, and the field work was brought to a close on May 24, 1940. During the course of operations, 105 Chihuahua deer-mice (*Peromyscus maniculatus blandus*) were marked and retrapped many times, and, in addition, 419 other small mammals comprising ten species were marked and observed.

It became evident soon after the work was begun that the trapping data would be adequate for a study of the home ranges and size of populations of nearly all of the small mammals on the trap areas. A technique for studying dispersal, successful in other areas, proved inadequate in this region (see p. 15), and the data here presented consequently deal largely with home-range size and features of population dynamics other than dispersal.

ACKNOWLEDGMENTS

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field assistant. Climatological data were furnished by the United States Weather Bureau. Publication was made possible by a grant from the Horace H. Rackham School of Graduate Studies.

THE MESQUITE ASSOCIATION

The principal vegetative features of the mesquite association of the Tularosa basin were described briefly but succinctly by Ruthven (1907: 495), who stated that ''the flora consists principally of the mesquite (with some crucifixion thorn).'' Dice (1930: 9) gave a more extensive description of the mesquite association and mentioned the presence of several species of *Atriplex* and a species of *Lepidium,* in addition to the plants mentioned by Ruthven.

The mesquite was by far the most abundant and most conspicuous plant on the area studied by me. In fact, all of the other plants could have been removed without altering materially the appearance of the vegetation. The habit of growth of the mesquite varied greatly. Some mesquites were small trees, ten to fifteen feet high. Others formed dense, shrubby masses of varying heights, and there were extensive areas of closely spaced, short, relatively straight mesquite stems, usually less than two feet in height. These last possibly were maintained in this condition by browsing cattle. Many mesquites and the crucifixion thorns were considerably affected by browsing. The clumps of mesquite alternated with barren areas on which there was little or no vegetation. The distance between clumps varied greatly. Around many of the clumps the accumulation of wind-blown sand and dust had built up mounds from a few inches to two or three feet high.

There were a few crucifixion thorns (*Koeberlinia spinosa*) and a few atriplex (*Atriplex canescens*). In April and May there were a few herbs, the most abundant being *Lepidium alyssoides*. Less numerous were *Astragalus nuttallianus, Verbena wrightii, Gilia pumila,* and *Sophia* sp.

In addition to the specimens of eleven species of small mammals caught, several species too large for our traps were known to frequent the areas trapped. Cottontails (*Sylvilagus audubonii minor*) were fairly numerous, and an occasional jack rabbit (*Lepus californicus texianus*) was seen. Coyotes (*Canis latrans*) were seen or heard on several occasions, and dogs traversed the area a few times. Deer tracks were seen once. Cattle were being ''grazed'' on the area when trapping was begun, but they had been removed before the end of the field work.

Food for the mammals of the mesquite association was limited during approximately the first half of the field period. The previous year's crop of mesquite beans and herbaceous seeds seemed to have been exhausted long before March. The only appreciable source of food appeared to be the bark of the mesquite. Many of the mesquite stems evidently had been gnawed near the ground. When a clump of mesquite was trampled to the ground, virtually all of the stems were gnawed clean of their bark before the next morning. After the spring growth began, the leaves and tender shoots of the mesquite and the fruit and foliage of *Lepidium alyssoides* probably provided the bulk of the food supply. On numerous occasions the small mammals carried these into the traps and there abandoned them in favor of the apparently more desirable sunflower seeds used as bait. The traps were baited liberally, and the Heteromyid rodents, after gorging themselves, filled their cheek pouches with sunflower seeds which they, when released, carried away and cached. After trapping had been under way for some time, the small piles of hulls indicative of these caches were numerous on the plots. Sometimes they were near mesquite clumps, sometimes in the barren areas intervening between the clumps. Possibly the caches in the open areas were safer from discovery by other individuals than those near the mesquites.

A great abundance of refuges and possible homesites was an important feature of the mesquite association. Nearly every clump of mesquite had one or more series of pocket-mouse or kangaroo-rat tunnels. Many of these, of course, were occupied by their builders or their descendants. Many others, however, were old tunnels that provided shelter for deer-mice, wood mice, or grasshopper mice. In addition to these multitudinous small holes and tunnels there were large abandoned holes that presumably had been dug by skunks and badgers. There also were some abandoned wood-rat dens and ground-squirrel holes. Because of this abundance of holes and tunnels, a small mammal that was released from a trap in any part of its home range usually was able to take refuge in a hole at the nearest mesquite clump.

The weather deviated slightly from the normal during the field period. The mean temperature for each month was slightly warmer than the average for these months (Table I). Precipitation was below normal in March and April, and considerably above normal in May.

TABLE I

CLIMATOLOGICAL DATA FOR ALAMOGORDO WEATHER STATION, NO. 1, FOR MARCH, APRIL, AND MAY, 1940

Month	Temperature F.						Precipitation		No. of Days Clear or Cloudy		
	Mean	Departure from Normal	Mean Max.	Mean Min.	Highest	Lowest	Total	Departure from Normal	Clear	Partly Cloudy	Cloudy
March	55.4	+2.5	69.6	41.2	82	24	0.10	−0.49	22	8	1
April	62.3	+2.2	77.8	46.8	88	32	0.01	−0.53	19	8	3
May	71.8	+3.1	86.2	57.5	94	48	2.09	+1.61	16	11	4

METHODS

The methods used in this study are in general those that have been developed in previous studies of home ranges and populations of small mammals (Blair, 1941*a*). Several modifications in technique, however, have been necessary because of local conditions.

PLOTS

Trapping was begun on a plot comprising 18.83 acres of mesquite association on March 4, 1940. Before the traps were set the plot was staked out in a grid pattern with the stakes forty-five feet apart. The traps were set out in lines that roughly followed the lines of stakes, although the traps were set at the stakes only where these were at or near the mesquite clumps, since small mammals of this association frequent these clumps and usually cross the intervening barren spaces only in moving from one clump to another. In general, a trap was set at each mesquite clump along a line of stakes, and two traps sometimes were used at large clumps. It soon became evident that the traps were more closely spaced than was necessary, and at the end of March alternate lines of traps were removed from the plot and used in another plot. After this change, plot A comprised 18.07 acres. The center of the second plot (plot B) was about one-half mile from the center of the first and comprised 17.69 acres (Fig. 1). This plot was staked out with the stakes forty-five feet apart in the lines, but with the lines ninety feet apart. The traps were spaced approximately the same on both plots and were maintained in this pattern until the end of trapping on May 24, 1940.

In outside dimensions plot A was 1215 feet long by 675 feet wide in March, and 1215 feet long by 648 feet wide in April and May. Plot B was 1044 feet long by 738 feet wide. These measurements were made from the outer traps on each side.

TIME INTERVALS

Both plots were divided into halves lengthwise, and one-half of each plot was trapped at a time. At the beginning, one-half of plot A was trapped for a week. After the first week, however, the trapping routine was altered slightly. With the new routine one-half of the plot was trapped for six days. On the seventh day the traps on only the outer half of this half-plot were set, and in addition the traps on the outer half of the other half-plot were set. Then the traps on the second half of the plot were set for the five following days. With this routine, in the course of twenty-five days each half of the plot was trapped for one five- and one six-day period. In addition, an outer one-fourth on each side of the plot was trapped on the three days intervening between the regular trap periods. This seemingly complicated, although really simple, trapping practice was devised to ensure the capture of each small mammal in as many parts of its home range as possible.

A small mammal often will enter the first trap that it encounters after leaving its domicile. This procedure was in part eliminated by leaving a

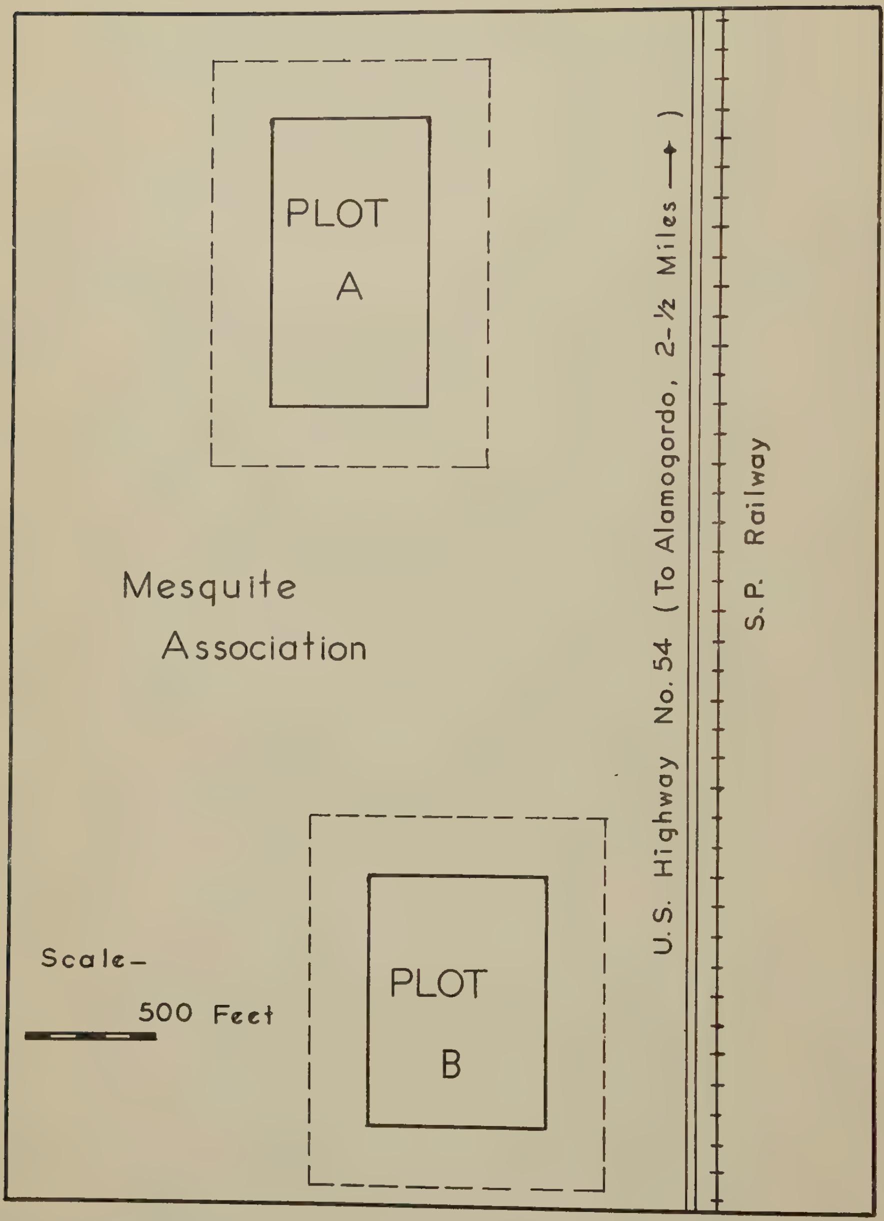

FIG. 1. Trapping plots in mesquite association near Alamogordo, New Mexico. Solid lines enclose areas on which traps were set. Broken lines enclose calculated areas from which mature male deer-mice were effectively trapped.

trap unset on the night following two consecutive captures of the same individual. There was always the possibility, however, that the animal would then be caught in the next adjacent trap. Therefore, the above described method for trapping different parts of the plot at different times was devised. The problem of localization of small mammal movements was recognized by Chitty (1937 : 44), who moved his traps in a rectangular pattern every night. Such a solution would have been physically impracticable in the present investigation.

Three different time intervals have been used in treating the various data:

''Trap month.'' This comprises the twenty-five-day period required to run through the trapping routine described above. One of these periods was completed in each month from March through May. In referring to these periods, only the month will be mentioned. The March period extended from March 4 to 29, the April period from April 1 to 25, and the May period from April 30 to May 24. The March period exceeded the others by one day, because of the full week of trapping at its beginning.

''Half month.'' This is the period for which calculations of populations and of concentrations were made. In the calculations the first twelve days of the ''trap-month'' period are considered one ''half-month'' period, and the last thirteen days are considered another. These periods will be mentioned as the first half and the last half of the respective months.

''Trap period.'' This period has been used only in summarizing the breeding records of the males. It includes the consecutive days on which one-half of the plot was being trapped, including the ''change-over'' day on which only the outer traps were set. There are seven days in each period, since there is an overlap of one day in consecutive periods because of the change-over day on which traps were set on only half of each half plot. This overlap is made necessary by the method used in keeping field notes on breeding.

TRAPPING METHODS

The traps were of a standard type with a pressed-wood box large enough to accommodate kangaroo rats and spotted ground squirrels (Blair, 1941*b*). Sunflower seeds were used as bait, and each trap was provided with cotton nesting material.

The traps were set shortly before dark in the evening and usually were run shortly after daylight. On a few of the coldest nights the traps were run between 10 P.M. and 1 A.M., and those that contained mammals were left unset after the captures had been released. All of the mammals were removed from the traps and were then marked, recorded, and released at the point of capture. The traps were left unset during the day because of the sun's heat.

The mammals were marked by the ear-punching and toe-clipping method, except for the pocket mice, on which only the toes were clipped.

METHOD OF CALCULATING HOME-RANGE AREA

A previously used method for calculating home-range areas (Blair, 1940*a*) was slightly modified, because here the traps necessarily were spaced irregularly instead of in a grid pattern. The outer limits of a given home range were drawn one-half the distance between the outer traps in which the animal was caught and the next adjacent traps. This is the same as was done when traps were set in a grid pattern. The only difference is that in this investigation the distance between any two pairs of traps seldom is the same.

Some animals will have ranges that extend beyond the limits of any plot. To eliminate most of the fractional ranges, the ranges of individuals that were caught only in a strip 180 feet wide around the outer edges of the plots were arbitrarily excluded from the computations of average home-range size. Some of the included ranges, however, extended to the outermost lines of the plots and may have continued beyond the edge of the plot. To have excluded them would have eliminated some of the largest ranges.

In computing home-range size, the trap month (see p. 7) has been used as the temporal basis for the calculation of home ranges. In the case of an individual who moved to a plot near the end of a trap month, the data for the last trap period (seven days) of that month have been added to those for the following month. Likewise, if an individual disappeared from a plot shortly after the beginning of a trap month, the data for the first trap period of that month have been added to those for the previous month. In no case have the data for more than one trap period been added to a trap month.

Many of the small mammals caught on the plots were taken so few times that their complete home ranges obviously were not learned. Consequently, all home ranges based on less than the arbitrary number of ten captures were eliminated as incomplete, except in the case of the rare species, where the data have been recorded for what they show of home-range size.

It was determined also whether or not each range calculated on ten or more captures ceased to increase in size before the last capture on which it was based. Only in the case of the female *Perognathus penicillatus* did all of the ranges cease to increase before the last capture (Table II). In the other classes, up to 20 to 40 per cent of the ranges increased slightly on the last time of capture. When the ranges that ceased to increase before the last capture were tabulated separately from those that increased with the last catch, however, there was no significant difference between the means. This, with the enormous range of variation in the number of captures necessary before a range ceased to increase (Table II), has led me arbitrarily to include in the calculations all ranges based on ten or more captures.

Haugen (MS), using a very different spacing of traps and a different method of computing home-range size, apparently found more uniformity in the number of captures necessary to show the complete home range of a cottontail than is evident in my data. It is not surprising to me to find such

TABLE II

Captures per Trap Month as Basis for Calculations of Average Home-range Size

Species	Sex	Ranges Based on Ten or More Captures		Ranges That Ceased to Increase Before Last Capture		Ranges That Increased with Last Capture of Month		Extremes	
		No.	Average Captures	No.	Average Captures	No.	Average Times Caught	Earliest Capture after Which Range Did not Increase	Latest Capture after Which Range Did not Increase
Peromyscus maniculatus blandus	♀	21	17.5	13	13.8	8	15.1	6	20
Peromyscus maniculatus blandus	♂	31	18.8	19	16.8	12	17.1	7	30
Perognathus penicillatus eremicus	♀	6	12.7	6	10.5	......	....	5	14
Perognathus penicillatus eremicus	♂	10	14.7	8	11.1	2	15.0	7	19
Dipodomys ordii ordii	♀	26	16.0	19	13.5	7	14.7	7	23
Dipodomys ordii ordii	♂	32	17.1	21	14.0	11	17.1	6	25
Dipodomys merriami ambiguus	♀	35	17.4	21	15.9	14	15.6	9	21
Dipodomys merriami ambiguus	♂	57	16.6	35	14.3	22	15.7	8	24

a great variation in the number of times that a small mammal must be trapped before its calculated home range ceases to increase. The area of a home range reflects many normal as well as what might be termed abnormal movements of its possessor. If an animal ranges widely on the first few nights of trapping, and, for some reason, less widely during later trapping, its calculated range may not increase after the first few times it is caught. If, on the other hand, the animal for one of many possible reasons does not venture far from its domicile during the first few nights of trapping and later ranges more widely, many captures may be necessary before its calculated range ceases to increase. In fact, its range may not be entirely defined in the limited time in which home ranges usually must be calculated.

In the statistical treatment of home-range size, standard errors rather than probable errors have been used. In comparing two means, a difference of 2.7 or more times its standard error is considered significant.

METHODS OF CALCULATING POPULATIONS AND CONCENTRATIONS

In calculating population density it has been kept in mind that many animals caught on a plot have ranges that extend beyond the limits of the trapping area. Therefore, one-half the width of the average home range has been added on each side of the plots to determine the area effectively trapped. Estimates of population density based on the area effectively trapped are consistently smaller and more accurate than those based merely on the area on which traps were set. One example should suffice to show the necessity for basing population estimates on the area effectively trapped. On plot B male Chihuahua deer-mice were being effectively trapped from a calculated area of 40.8 acres; but the traps were set out over an area of only 17.7 acres. On the same plot female deer-mice were being effectively trapped from a calculated area of 39.0 acres.

In computing concentration, the number of average home-ranges for each sex, age class, and species of mammal under consideration on the area effectively trapped is determined. Then, it is a simple matter to determine the concentration or average number of individuals per average home-range area.

AGE CLASSES AND BREEDING CRITERIA

Four age classes were recognized. Juveniles were those still completely in juvenile pelage. Subadults were those in which the juvenile pelage was being replaced by the first adult pelage. Young adults had the first adult coat, but they had not yet reached their full growth. Adults were fully mature in size. The age class determinations of the *Peromyscus* were more critical than were those of the other mammals because of my greater familiarity with all ages of these mice. For the purpose of summarization the juvenile and subadult classes in some parts of the text have been combined as an immature class. Young adults and adults have been combined as matures.

The location in the scrotum of testes large enough to be discerned by palpation was considered evidence of breeding activity of this sex. Scrotal testes were classified as large or medium in the field notes, but the two classes have been combined in summarizing the data. If the testes were indiscernible because of their small size, this was taken as evidence of sexual inactivity.

Two criteria of breeding of female Cricetidae were used. Late stages of pregnancy were evident from the external appearance of the females. The condition of the mammae indicated whether or not a female was suckling young. In female Heteromyidae a third clue to sexual activity was available, for a gelatinous plug forms in the vagina of these females following copulation.

SPECIES ACCOUNTS

Peromyscus maniculatus blandus

The Chihuahua deer-mouse was the main object of the study, and, consequently, the plots were laid out in an area that, in the fall of 1938, had the greatest deer-mouse population of any area of mesquite association in the central Tularosa basin. On plot A sixty-six deer-mice were trapped in three months, and on plot B forty-three were caught in two months.

HOME RANGES

The home ranges of neither males nor females showed any significant size variation from month to month. Therefore, the calculated home ranges for each of the three months have been combined to determine the mean monthly home-range size. Thirty-one home ranges of mature males caught an average of 18.8 times per month averaged 4.66 ± 0.33 acres. The largest range of a mature male comprised 9.92 acres in May, and the smallest comprised 2.03 acres in the same month. A mature male that was caught in all three months ranged over 3.53 acres in March, 3.22 acres in April, and 2.71 acres in May. Another male ranged over 8.00 acres in March, 5.76 acres in April, and 5.27 acres in May.

Twenty-one mature females caught an average of 17.5 times per month had an average home range of 4.10 ± 0.39 acres. The largest range of a mature female comprised 8.70 acres in April, and the smallest comprised 1.66 acres in March. The home range of one female that was on plot A during all three months comprised 5.43 acres in March, 5.51 acres in April, and 6.41 acres in May (Fig. 2).

There is no significant difference in the size of the male and the female home ranges. This is particularly interesting because in the subspecies *gracilus* (Blair, 1942) the home ranges of the males are significantly larger than those of the females.

The home ranges of both male and female *blandus* are significantly larger than those of *bairdii* of the blue-grass association in southern Michigan and of *gracilis* of the beech-maple association in northern Michigan. The difference between the means for mature male *blandus* and *gracilis* is 2.35 ± 0.43 acres. Between the means for mature female *blandus* and *gracilis*, the difference is 2.71 ± 0.42 acres.

The home ranges of both male and female deer-mice overlapped broadly in all of the months of trapping on both plots. This is indicative of widespread intrasexual tolerance among individual deer-mice. A similar relationship of individual home ranges existed among deer-mice of the subspecies *bairdii* and *gracilis*.

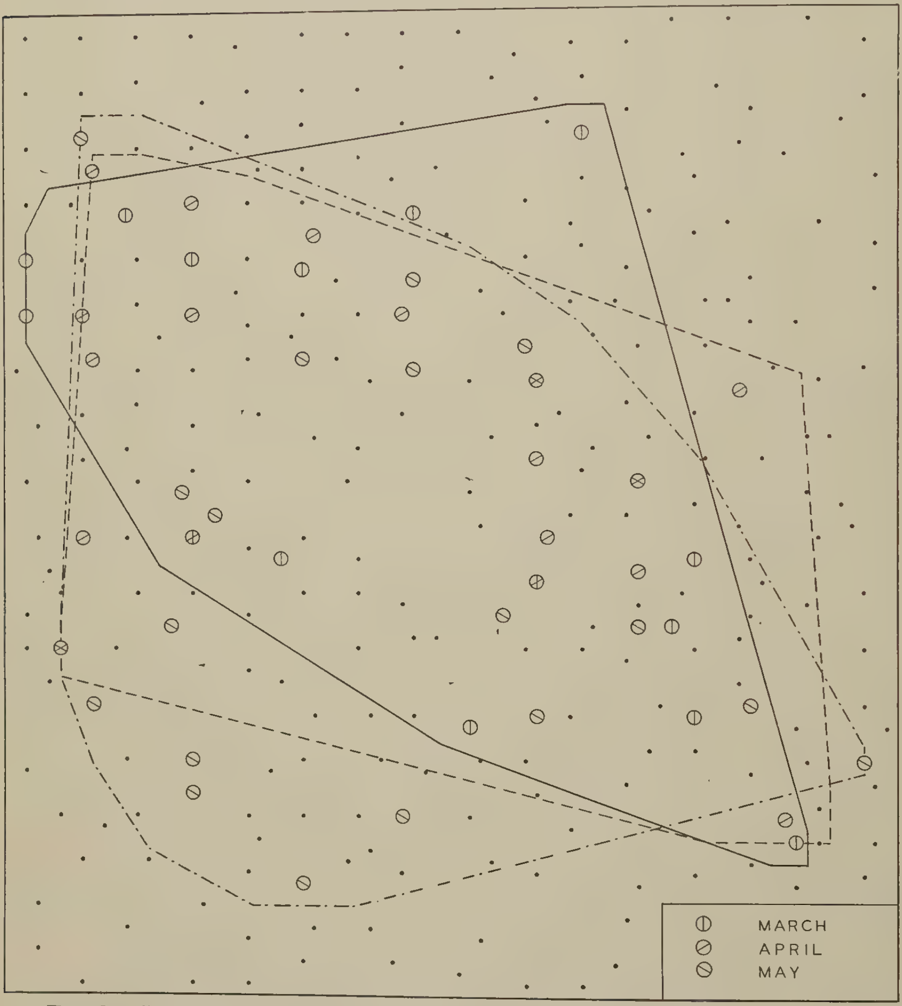

FIG. 2. Calculated home range of adult female deer-mouse in each of three trap months. Dots indicate traps. Circular symbols show traps in which this individual was caught during each trap month.

The month to month fluctuations in the home range of one mature female deer-mouse are shown in Figure 2. The general location of the range remained the same in all of the three months. Minor variations occurred, however, in each month. In March this animal ranged farther northeast than in any other month. In May it ranged farther southwest than in the other months. Otherwise, the limits of the monthly ranges differed little. It is interesting that several traps near the center of the range were never entered. The central area possibly was unattractive to this deer-mouse or was avoided for one of several possible reasons. The minor fluctuations in the home range of this individual are representative of those in the home ranges of the deer-mice of the mesquite association.

POPULATIONS AND CONCENTRATIONS

The average number of deer-mice per acre was about the same on both plots A and B. Consequently, the data for both areas have been combined to show the average population density of this species in the mesquite association at the time of the field work (Table III). The greatest density of mature females, an average of 0.27 individuals per acre, was in the second half of March. The maximum density of mature males was in the second half of April, an average of 0.35 individuals per acre. Most immature deer-mice were caught during the second half of March, when there was an average of 0.10 immatures per acre. The maximum density of all sexes and ages of deer-mice, an average of 0.67 deer-mice per acre, occurred in the second half of March. The drop to 0.41 deer-mice per acre in the first half of April can in large part be explained. In the last half of March a thoughtless hunter set some of our inactive traps on plot A, and before this was discovered several deer-mice entered them and died. The deer-mice of plot A recovered from the loss as other individuals moved to the plot, so that in the last three periods the population density was only a little less than the March maximum.

TABLE III

Average Populations of Deer-mice (*Peromyscus maniculatus blandus*) per Acre in Mesquite Association. Two Plots Combined

Class	March 4–16	March 17–29	April 1–12	April 13–25	April 30–May 11	May 12–24
Mature ♀	.14	.27	.14	.25	.20	.23
Mature ♂	.16	.30	.21	.35	.31	.33
Immature ♀	......	.05	.02	.03	.04	.04
Immature ♂	......	.05	.04	......	......	.03
Totals	.30	.67	.41	.63	.55	.63

The data on population density indicate that deer-mice were not numerous at the season that the trapping was being done. The five-toed and four-toed kangaroo rats and the pocket mouse were more numerous than the deer-mouse on plot A, and the last two species outnumbered it on plot B.

The average number of deer-mice per home-range area in each trapping period is listed in Table IV. The data for the two plots have been combined. The greatest concentration of mature females was in the second half of March, when there was an average of 1.09 females per home-range area. In only one other period, the last in April, was there an average of one or more mature females per home range. The least concentration of mature females was in the first half of April, when these averaged only 0.57 individuals per home-range area. The greatest concentration of mature males, an average of 1.64 males per home-range area, was in the last half of April. In three other periods there was considerably more than one male per home range. The least concentration of mature males, an average of only 0.76 males per home-range unit, was in the first half of March. The concentration of immature deer-mice always was slight. The greatest concentration of immature males and females combined was in the second half of March, when there was an average of 0.38 immatures per home range area.

TABLE IV

AVERAGE NUMBER OF DEER-MICE (*Peromyscus maniculatus blandus*) PER HOME-RANGE AREA IN MESQUITE ASSOCIATION. TWO PLOTS COMBINED

Class	March 4–16	March 17–29	April 1–12	April 13–25	April 30–May 11	May 12–24
Mature ♀	0.59	1.09	0.57	1.04	0.83	0.93
Mature ♂	0.76	1.41	0.96	1.64	1.41	1.52
Immature ♀	....	0.19	0.10	0.10	0.14	0.14
Immature ♂	....	0.19	0.15	....	....	0.10

BREEDING

A period of little or no breeding activity prior to the initiation of field work in early March was indicated by the fact that no immature deer-mice were caught before the third trapping period in March. In fact, it appears that the trapping was begun, as was planned, at approximately the time the spring breeding season began.

In March seven (64 per cent) of eleven mature females examined appeared to be pregnant or to be suckling young. In April seventeen (77 per cent) of twenty-two mature females appeared at some time to be pregnant or suckling. In May sixteen (67 per cent) of twenty-four mature females appeared pregnant or suckling. This does not mean that most of the females bore litters in every month, for only a few of the females were caught in all three months. One female that was under almost daily observation from the first to the last day of trapping bore three litters during the three months. Another that was observed in all three months apparently bore only one litter.

In the males, general sexual activity was indicated by the enlarged testes.

Only 75 per cent of the mature males were sexually active in the first March trap period. Mature males were all sexually active in all other periods except the third March and second and third May periods. In these last periods, respectively, 87, 87, and 77 per cent of the mature males were in breeding condition. Three subadult deer-mice were in breeding condition, and two juveniles had enlarged testes. Sexual maturity sometimes is attained before the juvenile coat is shed.

That deer-mice breed during the fall in the Tularosa Basin was indicated in 1938, when numerous immatures were caught between October 11 and December 7. There is evidence also that at least some breeding takes place during the summer. Dice (1930: 25) recorded three pregnant females taken at Alamogordo between July 9 and 12.

MOVEMENTS

Attempts to learn the distance of dispersal of deer-mice by the use of nest boxes failed, presumably because there were so many natural refuges available that the nest boxes did not appeal to the mice. The only records of movements of deer-mice from one home range to another were obtained when individuals moved from one of the trapping plots to another or when we set temporary lines beyond the limits of the plots. The amount of trapping that could be done outside of the plots was limited by the extensive nature of the trapping plots.

Such records of movements as are available are extremely interesting and give an indication of how far deer-mice sometimes may move. A young adult female appeared on plot A on May 1 and stayed until May 6. On May 8 she appeared on plot B, approximately 2000 feet away. On May 9 she was back on plot A, where she stayed until May 16. On May 18 she was back on plot B, where she stayed until May 23. On May 24, the last day of trapping, she was back on plot A. This female, therefore, made two round trips between the two plots, traveling a distance of about 2000 feet each time she moved from one plot to the other. Another female, an adult, appeared on plot A on April 15 and stayed until April 18. On April 20 she appeared about 2300 feet away on plot B, where she stayed until the end of trapping on May 24. Another adult female that moved away from plot A was caught in a trap about 1200 feet from where she previously had resided on the plot.

An adult male resident of plot A was caught in a trap about 1200 feet away. Another adult male appeared on plot A on March 19 and stayed until March 21. On March 23 he was caught in a trap about 1300 feet away. On March 24 he was back on the plot, but on March 29 he again was caught about 1300 feet away. This animal never was caught again.

The available data on movements indicate that journeys of more than one-third of a mile are not uncommon.

Peromyscus leucopus tornillo

Eight wood mice were trapped on plot A during April and May, whereas none had been caught there in March. Eleven wood mice were taken on plot B during April and May, but three of them previously had been recorded on plot A. The records of these animals not only provide interesting information about the movements of this species but give some indication of the size of the home range.

HOME RANGES

The size of the calculated home ranges of the mature wood mice that were caught seven or more times is listed in Table V. Most of these individuals probably were caught too few times to indicate the full extent of their home ranges. The largest home range of a mature male comprised 4.2 acres after the animal had been taken nine times. The largest home range of a female comprised 5.5 acres after she had been trapped twenty times. The size of her calculated home range failed to increase with the last three captures. It is interesting that one male had almost equal-sized calculated home ranges in both April and May, even though he was caught only seven times in April contrasted to fourteen times in May.

TABLE V

CALCULATED HOME RANGES OF ADULT WOOD MICE (*Peromyscus leucopus tornillo*) IN MESQUITE ASSOCIATION, APRIL AND MAY, 1940

Sex	Field No.	Month	Number of Times Caught	Last Capture on Which Size of Home Range Increased	Size of Home Range in Acres
♂	173	April	7	......	1.7
♂	262	April	7	6	3.3
♂	262	May	14	......	3.4
♂	414	May	9	......	4.2
♀	274	April	7	......	3.5
♀	139	May	20	17	5.5

The four home ranges of mature male wood mice averaged 3.1 acres. The two home ranges of mature females averaged 4.5 acres. Such data as are available suggest that, in the mesquite association, the wood mice have home ranges of approximately the same size as those of the deer-mice. The range of these wood mice in the mesquite association is much larger than the range of the subspecies *noveboracensis* in the oak-hickory association of southern Michigan (Burt, 1940: 26).

POPULATIONS AND CONCENTRATIONS

No wood mice were caught on plot A during the month of March. Two mature females and three mature males appeared on this plot during the first half of April. During this period there was an average of 0.13 wood mice per acre. Most of the wood mice soon disappeared, and in the three remaining half-month periods the average number of wood mice per acre varied from 0.05 to 0.10 individuals. On plot B there was one mature male, or an average of 0.06 individuals per acre, in the first half of April. In the three subsequent half-month periods there was an average of, respectively, 0.13, 0.11, and 0.19 individuals per acre. All of the wood mice were adults except one juvenile male taken on plot B in late May.

The trap records of both plots indicate that the wood mouse was relatively scarce in the mesquite association. In the Tularosa Basin the wood mouse reaches its greatest abundance in the grassy wash association. In the fall of 1938, by rough estimate, there were from ninety to one hundred wood mice per acre of grassy wash association near Tularosa (Blair, 1941*d*). In the spring of 1940, twenty-four wood mice were trapped from not more than ten acres of grassy wash association near Alamogordo in less than a week.

The greatest concentration of wood mice on plot A was in the first half of April, when there was an average of 0.22 females and 0.25 males per their respective, average home-range areas. On plot B the greatest concentration of females was in the last half of April and in all of May, when there was an average of 0.22 adult females per home-range area. The greatest concentration of male wood mice on plot B was in the last half of May, when there were 0.35 mature and 0.09 immature males per home-range area.

Of the sixteen wood mice recorded from the two plots, the males outnumbered the females three to one, twelve males and only four females.

BREEDING

The male wood mice for the most part gave evidence of sexual activity during the two months, April and May, that they were living on the plots. In April one of two males examined in the first trap period was sexually active, two of three in the second period, all of four in the third, and two of three in the fourth period were active. In May both of two males in the first period, both of two in the second, all of four in the third, and two of three in the fourth period were sexually active. In addition, one juvenile male in the fourth May period had enlarged testes, which indicates that breeding may begin before the juvenile coat is lost.

Of the five adult female wood mice, one appeared pregnant about the middle of April and again near the middle of May. Another appeared pregnant about the first of May. The three other females indicated potential breeding by their prominent mammae, but they were not trapped over a sufficiently long period to determine whether or not they became pregnant.

MOVEMENTS

No wood mice were taken on plot A during twenty-eight days of trapping in March. During April four adult males and two adult females appeared on this plot, and two more adult males appeared there during May. The first male was caught on April 1, and another appeared the next day. The first male stayed seven days on the plot, then disappeared. The second stayed only two days before disappearing. Two adult females were caught on plot A on April 7. One of these was last caught on plot A on April 13 and appeared on April 15 about one-half mile away, on plot B, where she remained until April 21. The other female stayed on plot A until April 13, then disappeared, but reappeared on May 7 and 8. An adult male appeared on plot A on April 10 and remained until April 13. This animal appeared on plot B, about one-half mile away, and remained until April 23 when it died in a trap. It is interesting that this male and one of the females disappeared from plot A on the same date and that both appeared on plot B two days later. Possibly, they traveled together in moving from one plot to the other. Another adult male appeared on plot A on April 16 and stayed until April 26. One adult male appeared on plot A on May 10 and stayed until May 12. This animal appeared on plot B, about one-half mile away, on May 14 and stayed until the end of trapping. A young adult male apparently passed through plot A on May 16, for it was taken there only once.

One immature and seven adult wood mice moved to plot B in addition to the three adults that moved there from plot A. No wood mice apparently resided on the plot when trapping was begun; none was taken there during the first six days of April. An adult male transient was recorded on April 7. An adult male appeared on April 10 and stayed until May 16. An adult female appeared on April 13 and stayed until April 25. An adult male was first taken on April 15 and was last taken on May 20. Another adult male appeared on May 1 and disappeared after May 3. One adult female was on the plot from May 10 to 12. An adult male appeared on May 19 and remained until the end of trapping. An immature male appeared on plot B on May 20 and disappeared after May 22.

The trap records of the wood mice seem to indicate nomadic tendencies for this species in the mesquite association during April and May. The movements of the deer-mice to the plots and away from them were not dispersal movements of young animals. Rather, they were, with one exception, movements of fully mature breeding animals that appeared to be at least six months or more of age. The movements to both plots represented invasion of areas that were, so far as known at that time, uninhabited by the species. Some of the wood mice settled there more or less permanently. Some others established residence for rather short periods of time. Others merely passed through the plots. The movement of three individuals from one plot to the other indicates that movements of as much as one-third to one-half mile are not uncommon in this species. The fact that in all three interplot movements one night elapsed between the capture of the mice on plot A and their capture on plot B gives an indication of the speed of travel of the wood mice. The fact that one male and female moved from one plot to the other at the same time possibly indicates that wood mice sometimes travel in pairs in moving from one area to another.

Citellus spilosoma major

No attempt was made to mark and study the movements of the spotted ground squirrels of the mesquite association. These animals, being diurnal, got into the traps only occasionally. Those that were caught were removed from the area for a study of their pelage color. Ten individuals, of which seven were males and three were females, were trapped on and near plot A. None was caught on plot B. It seems unlikely that all of the ground squirrels living on plot A were caught, nor is it safe to assume that none was living on plot B. The available data, however, point to a greater concentration of ground squirrels on plot A than on plot B.

A female that was trapped on the White Sands gave birth to six young on May 14.

Perognathus penicillatus eremicus

This pocket mouse was fairly numerous on both plots. Sixty-seven individuals were caught on plot A, and thirty-three were recorded on plot B. The trap records provide information about the size of the home ranges, population density, and breeding season of these pocket mice.

HOME RANGES

So many pocket mice died of cold in the traps that only a small fraction of all those on the plots were caught a sufficient number of times to indicate the size of their home ranges. Ten ranges of mature males, based on an average of 14.7 catches each per month, averaged 2.72 ± 0.48 acres. The largest home range of a male comprised 5.54 acres in May; the smallest, 1.23 acres in the same month. The home ranges of six mature females that were caught an average of 12.7 times per month averaged 1.09 ± 0.14 acres. The largest home range of a female comprised 1.43 acres in May; the smallest 0.52 acres in the same month. The home ranges of the mature male pocket mice averaged significantly larger than those of the females, for the difference in the means is 1.63 ± 0.50 acres.

With eleven mature females on plot A in March, the known home ranges of three overlapped rather broadly. In two instances two different females were in the same trap on consecutive nights. In April the nine known home ranges and fractions of home ranges of mature females on plot A failed to overlap, with one exception in which there was a slight overlapping of two ranges. In May, on the same plot, there were twelve mature females, and in only one case was there even a slight overlapping of home ranges. On plot B there was no overlapping of the six known ranges of mature females in April, nor of the ranges of eleven females in May. It appears probable that the home ranges of the mature female pocket mice are mutually exclusive during the breeding season. In March, when there was little evidence of sexual activity, the known home ranges of females did overlap to some extent. In April and May, after the onset of the breeding season, the ranges of females showed virtually no overlap.

The home ranges of the mature male pocket mice broadly overlapped during every month of trapping on the two plots. It seems evident, therefore, that the home ranges of the males are not mutually exclusive either in or out of the breeding season. The home ranges of the males broadly overlapped those of the females.

POPULATIONS AND CONCENTRATIONS

The population of pocket mice on plot A was lower in the first half of March than at any other time during the study. In this period there were seven males and three females or an average of 0.29 individuals per acre. The population of pocket mice on plot A reached its greatest density in the first half of May, when there were ten females and fifteen males, an average of 0.77 individuals per acre. On plot B the smallest population was recorded in the first half of April, when there were three males and one female, an average of 0.14 individuals per acre. The largest population on plot B was found in the last half of May, when there were nine females and thirteen males, an average of 0.70 individuals per acre.

The greatest concentration of female pocket mice was on plot A in the last half of March, when there was an average of 0.41 individuals per average home-range area. The concentration of females on plot A was at a low of 0.11 individuals per home-range area in the first half of March and the last half of April. On plot B the greatest concentration of females was in the last half of May, when there were 0.35 females per average home-range area. The least concentration of females on plot B, an average of 0.04 individuals per home-range area, was in the first half of April. It is evident that at no time during the trapping were the female pocket mice greatly concentrated on either plot, for there always was an area equivalent to at least two or more average home ranges available for every female recorded on the plots.

The concentration of adult male pocket mice on plot A averaged less than one individual per home-range area only in the first half of March, when there was a low of 0.52 males. In the last half of March the concentration was up to 1.04 males per home-range area, and in the first half of April it reached a high of 1.38 individuals. On plot B the concentration of males was low during April, with averages for the half months of, respectively, 0.24 and 0.55 males per home-range area. In May the concentration of males on plot B averaged 1.02 individuals per home range. It is obvious that the male pocket mice were consistently more concentrated on both plots than were the females, for after the middle of March on plot A and after the first of May on plot B there was an average of at least one male per home-range area.

Cahalane (1941: 62) calculated that there was an average population of

9.9 individuals of this species in an area of mesquite and grass in the San Simon Valley, Arizona, in late August, 1933. He pointed out, however, that the size of the home range, which is necessary for determining the area effectively trapped, was not known. If the area effectively trapped in his work is determined from the average home ranges on my plots, his average of 9.9 individuals per acre drops to 3.2 per acre.

Of the total pocket mice trapped on my two plots, thirty-nine (39 per cent) were females and sixty-one (61 per cent) were males. The excess of males is accounted for in part by the fact that, because of their larger home ranges, males were caught from greater distances from the actual trapping areas than were females. This error is, in large part, eliminated by calculating the sex ratio from the average number of individuals of each sex per acre effectively trapped. On this basis the female pocket mice comprised 44.3 per cent and the males 55.7 per cent of the average number of individuals caught per acre. The remaining discrepancy between the number of males and females probably is accounted for by the greater wandering tendency of the males.

BREEDING

The testes of the male pocket mice examined in March were mostly small or medium in size, which indicated that the breeding season was then just beginning. In April and May the testes of the males examined were mostly large and indicative of general reproductive activity. No sexual activity of females was noted until the trap period of April 19 to 25, when one of four females examined appeared to be pregnant. There was increased evidence of sexual activity of the females in May. Among twenty-three females examined in May, nine had vaginal plugs that indicated recent copulation, and five others appeared to be pregnant.

Perognathus flavus flavus

This little pocket mouse was one of the rarest species of the mesquite association, and only three individuals, of which one previously had been released near by, were recorded on the two plots. An adult male was taken on May 11 on plot B, and an adult female was taken 180 feet distant on May 15. The condition of the mammae of the female indicated that she recently had suckled young, and the enlarged testes of the male showed that he was in breeding condition.

An adult female that had been trapped on the White Sands was released near plot A. This individual later was caught on plot A, about 1200 feet from the point of release. Ten days after the first recapture it again was trapped on plot A about 400 feet from the point at which it first was retaken.

Dipodomys ordii ordii

The five-toed kangaroo rat was the most abundant species of mammal on plot A, where eighty-four were trapped in three months. On plot B, where only twenty-eight were caught in two months, this species was exceeded in abundance by the four-toed kangaroo rat, by the deer-mouse, and by a pocket mouse.

HOME RANGES

As the home ranges of the male five-toed kangaroo rats did not vary significantly from month to month, the home ranges for the three months have been combined to show the average size of the male ranges. Thirty-two home ranges of mature males caught an average of 17.1 times per month averaged 3.41 ± 0.25 acres.

The largest home range of a male comprised 6.93 acres in May, and the smallest comprised only 0.51 acres in April. The amount of month to month variation in the home ranges of individual males is shown by the records of four males that were caught frequently in all three months. One male had a calculated range of 3.42 acres in March, 3.44 acres in April, and 2.79 acres in May. Another had a range of 3.63 acres in March, 3.82 acres in April, and 2.73 in May. A third male had a range of 1.18 acres in March, 2.73 acres in April, and 2.61 acres in May. A fourth had a range of 4.59 acres in March, 3.50 acres in April, and 5.02 acres in May.

The three home ranges of mature females recorded for March were so much smaller than most of those for April and May that they have been treated separately. The March ranges averaged 1.09 ± 0.36 acres, and the twenty-three ranges for April and May averaged 3.29 ± 0.47 acres. The difference between the two means is 3.7 times its standard error, a difference of 2.20 ± 0.59 acres. The mature females whose ranges were calculated were caught an average of 16.0 times each month.

The largest home range of a mature female comprised 10.41 acres in May. The smallest comprised 0.32 acres in March. This is indicative of considerable individual variation in home-range size. The amount of month-to-month variation in the size of the home range of an individual female is shown by the ranges of one female that was caught in all three months. In March the range of this animal comprised only 0.32 acres, in April, 2.27 acres, and in May, 3.38 acres. The average home ranges of the females in April and May did not differ significantly in size from those of the males.

The home range of each male and female usually was in the same general place so long as the animal was on the plot. The fluctuations in size of range generally were due to fluctuations in the limits of the range, rather than to an actual change of range from one locality to another.

The home ranges of breeding animals of both sexes broadly overlapped the ranges of other members of both sexes. In spite of the fact that several individuals of the same sex were living in the same general area, some parts of both plots were unoccupied by this species. The concentration of these kangaroo rats in certain places, while near-by areas were untenanted, was most striking on plot B. Most of the five-toed kangaroo rats were concentrated on roughly one-third of the plot, but approximately one-half of the plot had none of this species.

POPULATIONS AND CONCENTRATIONS

Five-toed kangaroo rats were much more numerous on plot A than on plot B. Consequently, the average populations of this species per acre on the two plots have been treated separately (Table VI). On plot A the greatest number of mature females occurred in the last half of April, when there was an average of 0.51 individuals per acre. On plot B the maximum number of mature females was present in the last half of May, when there was an average of only 0.25 per acre. Thus, the maximum population of mature females on the former plot was approximately twice that on the latter.

TABLE VI

Average Populations of Five-toed Kangaroo Rats (*Dipodomys ordii ordii*) per Acre on Each of Two Plots in the Mesquite Association

Plot A	March 4–16	March 17–29	April 1–12	April 13–25	April 30–May 11	May 12–24
Mature ♀	0.24	0.20	0.35	0.51	0.40	0.40
Mature ♂	0.46	0.49	0.71	0.63	0.58	0.61
Immature ♀	....	....	0.03	0.03	0.03	0.11
Immature ♂	....	....	0.03	0.03	0.05	....
Totals	0.70	0.69	1.12	1.20	1.06	1.12
Plot B						
Mature ♀	Not Trapped		0.16	0.14	0.19	0.25
Mature ♂			0.14	0.14	0.14	0.16
Immature ♀			....	....	0.05	0.03
Immature ♂			....	....	0.08	0.03
Totals			0.30	0.28	0.46	0.47

Mature males on plot A were most abundant in the first half of April, when there was an average of 0.71 individuals per acre. On plot B the maximum number of mature males occurred in the last half of May, when there was an average of only 0.16 individuals per acre. It is evident that mature males were from four to five times as abundant on the former as on the latter plot in every period in which the two were compared.

The unequal proportions of males to females on the two plots are extremely interesting. On plot A there always were more males than females, and in three of the periods males exceeded females two to one. On plot B the females equaled or exceeded the males in abundance in every trapping period. It is evident that in this species at least marked local fluctuations in the sex ratio may occur. The condition observed here is unusual in small mammals. Generally, the sex ratio is even, or the males are slightly more numerous than the females.

Immature five-toed kangaroo rats were not numerous on the plots. The greatest number of immatures of both sexes were on plot B during the first half of April, when there was an average of 0.13 immatures per acre. The greatest average of five-toed kangaroo rats of all sexes and ages occurred on plot A during the last half of April, when there was an average of 1.20 individuals per acre. On plot B the maximum was reached in the last half of May, when there was an average of 0.47 five-toed kangaroo rats per acre.

The average number of five-toed kangaroo rats of the several sex and age classes per home-range area is shown in Table VII. The greatest concentration of mature females was on plot A in the last half of April, when there was an average of 1.67 females per home-range area. Mature males were most concentrated on plot A during the first half of April, when there was an average of 2.43 individuals per home-range unit. Immatures were not very heavily concentrated, for the average was no more than a small fraction of one individual per home-range area.

TABLE VII

AVERAGE NUMBER OF FIVE-TOED KANGAROO RATS (*Dipodomys ordii ordii*) PER HOME-RANGE AREA ON EACH OF TWO PLOTS IN THE MESQUITE ASSOCIATION

Plot A	March 4–16	March 17–29	April 1–12	April 13–25	April 30–May 11	May 12–24
Mature ♀	0.26	0.22	1.14	1.67	1.32	1.32
Mature ♂	1.58	1.67	2.43	2.16	1.98	2.07
Immature ♀	....	....	0.09	0.09	0.09	0.35
Immature ♂	....	....	0.09	0.09	0.18	....
Plot B						
Mature ♀	Not Trapped		0.54	0.45	0.63	0.81
Mature ♂			0.46	0.46	0.46	0.56
Immature ♀			....	....	0.18	0.19
Immature ♂			....	....	0.28	0.09

BREEDING

The breeding season of *ordii* evidently started shortly before trapping was begun in early March, for no young of the year were caught before early April. That breeding still was in progress when trapping was discontinued in late May was shown by the sexual condition of the animals living on the plots. Fall and early winter breeding of this species in southern New Mexico is indicated by a breeding male collected on October 11, 1938, also by records published by Bailey (1931: 268). Whether breeding continues throughout the summer or is broken into spring and fall periods is not yet known.

In each trapping period 84 to 100 per cent of all of the males on the plots were sexually active, as indicated by their enlarged testes. Immature as well as adult males are included in these calculations, for several of the juvenile and subadult males had large testes in the scrotum.

Three criteria of sexual activity of female kangaroo rats were used. Late stages of pregnancy were indicated by the distended abdomen. Females that were suckling young indicated this fact by the condition of their mammae. In addition, a gelatinous plug that forms in the vagina of female kangaroo rats following copulation was an excellent indicator of sexual activity. This last phenomenon previously has been described for the species *Dipodomys spectabilis* by Vorhies and Taylor (1922: 17).

At some time during March eight (89 per cent) of the nine females examined either were suckling young, apparently pregnant, or exhibited a vaginal plug. In April twenty-nine (94 per cent) of the thirty-one females examined at some time exhibited one or more of the criteria of sexual activity. In May twenty-nine (88 per cent) of the thirty-three females examined were at one or more times pregnant, lactating, or had recently copulated. Several juvenile and subadult females were found with vaginal plugs which, with the breeding of immature males, proves that the five-toed kangaroo rats like many other rodents become sexually active long before they are fully grown.

Two females that were caught frequently in all three months apparently gave birth to, respectively, three and four litters. The first of these females had a vaginal plug in the first March period and was pregnant, near term, in the first April period. In the third April period she again appeared pregnant, and in the first May period she was suckling young. In the third May period she again appeared to be pregnant. The other female was suckling young when first caught in the second period of March. She had a vaginal plug in the third and fourth March periods, and appeared pregnant in the first April period. She again appeared pregnant in the last April period and on April 23 gave birth, in a trap, to two young. She again appeared pregnant in the last May period. Eleven of the females that were caught in two months gave evidence of copulating at least twice during that period, and in several of these cases two litters evidently were produced.

In some of the females the vaginal plug persisted for a long time without any discernible evidence of pregnancy. One female had a recent plug when first examined in the first April trap period, and she subsequently had a plug in every trapping period until the work was discontinued on May 24. It is not certain that the same plug persisted throughout this entire period. Rather, it seems quite possible that copulation took place several times. Other females had vaginal plugs that persisted almost as long as in this example. In some cases a plug persisted for a considerable period, and then was replaced by an obviously later one.

All of my observations of breeding of the five-toed kangaroo rat in southern New Mexico indicate a high rate of increase of the species there. There is a breeding season of at least three months in the spring, and there is at least some reproduction in the fall. In the spring most mature females produce at least two, and sometimes more, litters of young. Furthermore, the young of the year begin breeding long before they reach adult size, thus augmenting greatly the multiplication of the species during the spring season. The rapid rate of reproduction indicated for this species contrasts markedly with the "surprisingly low rate of reproduction" for which Vorhies and Taylor (1922: 18) found evidence in another species (*Dipodomys spectabilis*).

MOVEMENTS

Only one record of the distance of a movement of a five-toed kangaroo rat, other than within the home range, was obtained. A subadult female appeared on plot B on May 8. This animal appeared on plot A, some 2000 feet distant, on May 9 and stayed until May 11, after which it was not taken again.

Dipodomys merriami ambiguus

The four-toed kangaroo rat was the most abundant species of mammal on plot B, where seventy-four individuals were caught in two months. On plot A, where seventy were trapped in three months, it was exceeded in abundance only by the five-toed kangaroo rat (*Dipodomys ordii*).

HOME RANGES

The home ranges of mature males of this species, like those of the five-toed species, did not vary significantly in size from month to month. Consequently, all of the home ranges of mature male *merriami* have been combined to determine the average home-range size. The average of fifty-seven home ranges of males, based on an average of 16.6 captures per month, was 4.07 ± 0.24 acres. The largest home range of a male comprised 8.78 acres in May. The smallest comprised 1.18 acres in April.

Usually, each of these kangaroo rats, like individuals of most of the other species on the plots, remained in one general locality, although the exact limits of each calculated home range fluctuated somewhat from month to month. Calculated home ranges are available for four males in each of the three months of trapping. The male that exhibited the greatest monthly difference in home-range size had a calculated range of 6.44 acres in March, 3.14 acres in April, and 1.95 acres in May. His range in March was 4.49 acres larger than the area over which he was known to have ranged in May. The male with the least monthly variation in home-range size had a calculated range of 2.41 acres in March, 2.83 acres in April, and 3.08 acres in May. His range was only 0.67 acres larger in May than it was in March.

The home ranges of mature females, like those of the five-toed species, averaged considerably smaller in March than in the two following months. Therefore, the March home ranges have been treated separately from the others. In March eight home ranges, based on an average of 14.5 captures, averaged 1.94 ± 0.22 acres. In April and May twenty-seven home ranges, based on an average of 18.3 captures, averaged 3.88 ± 0.51 acres. The March ranges were significantly smaller than those for April and May, the difference in the means being 1.94 ± 0.55 acres. Although the number of March ranges was small for both species of kangaroo rats, the parallel condition in the two species lends weight to the evidence that those females ranged over smaller areas in March than in the later months. Data now available do not suffice to account for the small size of the female home ranges in March.

The largest home range of a female comprised 12.52 acres in April. The next largest comprised 11.81 acres in the same month. The smallest comprised only 1.17 acres in May. The range of variation in home-range size is greater than in any other small mammal of which I know. The third largest range comprised only 5.89 acres. The two largest ranges are puzzling, because they so greatly exceed the other female ranges. The large calculated range of one of these females possibly was due to a shift in range, as the animal never again was taken on the particular part of the plot on which it first was caught. No such possible explanation can be given for the other large range, and it seems probable that this merely was an unusually wide-ranging individual.

There was no significant difference in size between the home ranges of the male *merriami* and the April–May ranges of the females. Neither was there any significant difference between the ranges of the *merriami* and those of the species *ordii*.

Four immature females, caught an average of 14.2 times each, had an average home range of 4.56 acres. No immature males were caught a sufficient number of times to indicate the size of their home ranges.

The home ranges of both males and females broadly overlapped others of the same sex on both plots in every month of trapping.

POPULATIONS AND CONCENTRATIONS

The four-toed kangaroo rat was approximately equal in abundance on both of the trapping plots. Consequently, the population data for the two plots have been combined to show the average number of individuals per unit area at that season of the year (Table VIII). The greatest density of mature females was in the last half of May, when there was an average of 0.45 indi-

TABLE VIII

AVERAGE POPULATIONS OF FOUR-TOED KANGAROO RATS (*Dipodomys merriami ambiguus*) PER ACRE IN MESQUITE ASSOCIATION. TWO PLOTS COMBINED

Class	March 4–16	March 17–29	April 1–12	April 13–25	April 30–May 11	May 12–24
Mature ♀	0.24	0.27	0.33	0.35	0.39	0.45
Mature ♂	0.39	0.46	0.56	0.50	0.47	0.46
Immature ♀	....	....	....	0.01	0.07	0.11
Immature ♂	....	....	0.02	....	0.03	0.06
Totals	0.63	0.73	0.91	0.86	0.96	1.08

viduals per acre. The greatest density of males was in the first half of April, when there was an average of 0.56 males per acre. The number of immatures of this species per acre was low in every period, and none was taken in either March period. The greatest number of immatures was taken in the last half of May, when there was an average of 0.11 immature females and 0.06 males, or 0.17 of both sexes combined per acre. The greatest density of all age and sex classes of the species likewise was found in the last half of May, when there was an average of 1.08 four-toed kangaroo rats per acre.

The average number of the several classes of this species per their respective, average home-range areas is shown in Table IX. Mature females reached a maximum concentration of 1.74 individuals per home-range area in the last half of May. Mature males were more concentrated than mature females and reached a maximum concentration of 2.32 individuals per average home-range area in the first half of April. Immatures never were very much concentrated. The greatest concentration of immature females was in the last half of May, when there was an average of 0.50 individuals per home-range area. Immature males reached a maximum of 0.26 individuals per home-range area in the same period.

TABLE IX

AVERAGE NUMBER OF FOUR-TOED KANGAROO RATS (*Dipodomys merriami ambiguus*) PER HOME-RANGE AREA IN THE MESQUITE ASSOCIATION. TWO PLOTS COMBINED

Classes	March 4–16	March 17–29	April 1–12	April 13–25	April 30–May 11	May 12–24
Mature ♀	0.46	0.52	1.30	1.34	1.49	1.74
Mature ♂	1.58	1.88	2.32	2.06	1.90	1.86
Immature ♀	....	....	....	0.05	0.33	0.50
Immature ♂	....	....	0.05	....	0.10	0.26

Monson and Kessler (1940: 40) counted *merriami* dens on one-acre plots in creosote bush association in Arizona and by excavating on some of them found that there was an average of one *merriami* to 4.11 dens. There were an average of 31.3 dens per acre, which, by their rat–den ratio, gives an average of 7.6 individuals per acre. Their data, however, are not comparable with mine because their averages apparently cover a period of more than a year, and, consequently, take no account of seasonal fluctuations in abundance, nor, so far as I can see, in seasonal differences in the rat–den ratio.

Cahalane (1941: 62) calculated that there was an average of 3.2 individuals per acre in an area of mesquite and grass in the San Simon Valley, Arizona, in late August, 1933. If the area, however, that he was trapping effectively is calculated from my home-range data, his average drops from 3.2 to 0.9 individuals per acre.

BREEDING

The breeding season of *merriami* did not begin until early March, after trapping was initiated. The first immature was not taken until the first trap period in April, and no other was taken until after the middle of that month. Breeding still was in progress when field work was discontinued in late May.

The breeding season of the *merriami* began somewhat later than that of *ordii.* In eight (73 per cent) of eleven males examined in the first period in March, the testes were sufficiently large to be discernible. In only two individuals, however, had the testes attained the large size characteristic of peak breeding activity. Evidence of sexual activity of males increased in the second March period, when 79 per cent were in breeding condition. In subsequent periods from 86 to 100 per cent of the males examined in each trap period were sexually active.

Not only did the breeding season begin later than in *ordii,* but there was never so large a fraction of the females on the plots breeding at one time as in the other species. In March only three (27 per cent) of the eleven females examined evidenced sexual activity. Two of these had vaginal plugs on March 20, and the other had a vaginal plug on March 23. This is in considerable contrast to the 89 per cent of breeding female *ordii* in March. In April eighteen (50 per cent) of thirty-six female *merriami* at some time showed evidence of breeding, in contrast with the 94 per cent of breeding female *ordii* in the same month. In May thirty-three (67 per cent) of the forty-nine *merriami* females examined were at some time sexually active, compared with 88 per cent of active *ordii.*

Eleven females indicated by their vaginal plugs that they copulated at least twice during the period of trapping. There was evidence that at least three of these females gave birth to two litters. Most of the juvenile and subadult females appeared with vaginal plugs. Likewise, most of the juvenile and subadult males developed large testes before they were grown. This indicates that, like *ordii,* the species *merriami* becomes sexually active long before it matures in size.

Specimens preserved in alcohol in the collection of the University of Michigan Museum of Zoology indicate that breeding continues well into the summer and that there is at least some breeding in the fall. Among twelve males from the same area, dated July 3 to 15, 1927, nine (75 per cent) were sexually active when killed. Of twelve females, six (50 per cent) were sexually active when killed. Two of these females contained two embryos each, three evidently had recently suckled young, and one young adult had a vaginal plug. Of two males from Carlsbad, Eddy County, dated August 12 to 16, 1926, an adult had large testes; a half-grown male had small. Of two females from the same place and collected at the same time, an adult was not sexually active, but a half-grown female had a vaginal plug. Among seven specimens collected near Alamogordo on October 11, 1938, two adult males were sexually active; three adult males and two adult females showed no sign of breeding. All of the available evidence indicates that *merriami,* like *ordii,* has a much more rapid rate of reproduction than Vorhies and Taylor (1922: 18) found in *spectabilis.*

MOVEMENTS

Only two records of the distance of movements of four-toed kangaroo rats from one area to another were obtained. A young adult male was caught once on plot B on May 11. This animal appeared about 2000 feet away on plot A on May 18, where it was taken only once before it disappeared. Another young adult male appeared on plot A on May 13 and stayed until May 20. On May 22 it was caught about 2700 feet away on plot B, but it disappeared after being caught there once.

Onychomys leucogaster ruidosae

The grasshopper mouse was rare and unevenly distributed in the mesquite association. Only five individuals were taken during three months of trapping on plot A, and none was taken in two months on plot B. The trap records of the five grasshopper mice, however, provide some very worth-while information about the species.

HOME RANGES

Only one grasshopper mouse was trapped more than ten times in a month, and for only three individuals did the size of the known home range fail to increase with the last capture of the month (Table X). Therefore, some of the calculated home ranges probably represent only a fraction of the actual range. Several nights often elapsed on which one or another of these grasshopper mice was not caught. This possibly indicates that their ranges extended for considerable distances outside of the plot. Consequently, the calculated ranges should be considered only as minimum ranges. The largest known range was that of a subadult male and comprised 11.4 acres in

TABLE X

Calculated Home Ranges of Grasshopper Mice (*Onychomys leucogaster*) March, April, and May, 1940

Ranges that increased with last capture possibly incomplete

Age	Sex	Field No.	Month	Number of Times Caught	Last Capture on Which Size of Home Range Increased	Size of Home Range in Acres
Ad.	♂	41	April	9	6	3.8
Ad.	♂	41	May	16	14	4.8
Subad.	♂	68	March	8	......	11.4
Yg. ad.	♂	68	April	8	7	6.6
Yg. ad.	♂	68	May	7	......	8.0
Ad.	♀	71	March	6	......	1.5
Ad.	♀	71	April	6	......	2.1

March. As a young adult in April and May this individual had notably smaller known ranges of 6.6 and 8.0 acres, respectively.

The average of the two calculated monthly home ranges of the young adult male and of the two monthly ranges of an adult male was 5.8 acres. The average of the two monthly home ranges of an adult female was 1.8 acres. Although the numbers are small the great disparity between the mature male and female averages suggests a possible sexual difference in the size of the home range.

POPULATIONS AND CONCENTRATIONS

The adult male and female grasshopper mice and the one subadult and two juveniles that were trapped on plot A in March possibly represented the parents and the remnants of two of their litters. The two juveniles were not recorded on the plot after March, which possibly indicates dispersal of these individuals. The adult female disappeared after April 16. Whether she fell victim to a predator or moved away I do not know. The original adult male and the young adult that matured there remained on the plot until the end of trapping.

The population per acre was small for all of the periods. The greatest density of population was in the two March periods, in each of which there were only 0.08 individuals per acre. The scarcity of these grasshopper mice on plot A and their absence from plot B indicate that the species was relatively rare and unevenly distributed in the mesquite association in the spring of 1940.

The concentration of the several sex and age classes of grasshopper mice was low in all trapping periods. The greatest concentration was of immature males in the first half of March, when there were 0.38 immature males per average home range. There was no more than a small fraction of one individual per average home range in any sex or age class.

BREEDING

The subadult and juvenile grasshopper mice trapped in March indicated that young were born in January and February. Examinations of the adult female indicated that she bore a litter about the first of April. No young of this litter were caught, and it is possible that they died from neglect while the mother was in a trap. The large testes of the adult male showed sexual activity during the entire trapping time, from early March to late May. Sexual activity of the one individual first caught when a subadult was indicated by enlarged testes while still in juvenile pelage. It still was sexually active when last caught in late May. Bailey (1931: 141) recorded pregnant females of this species from San Pedro and Socorro taken on July 10 and August 19, respectively. Therefore, it seems probable that the breeding season extends at least into late summer.

Onychomys torridus torridus

The scorpion mouse was found on both of the trapping plots. Nine specimens were caught during three months of trapping on plot A, and five were captured in two months on plot B.

HOME RANGES

The calculated home ranges of the scorpion mice are given in Table XI. The largest home range of a mature male comprised 12.4 acres; the smallest, only 3.1 acres. The largest range was about two-thirds as large as the entire trapping area. The male that had the maximum range of 12.4 acres in March had a range of only 6.9 acres in April. The four monthly ranges of mature males averaged 7.8 acres.

TABLE XI

Calculated Home Ranges of Scorpion Mice (*Onychomys torridus*) in Mesquite Association, March, April, May, 1940

Age	Sex	Field No.	Month	Number of Times Caught	Last Capture on Which Size of Home Range Increased	Size of Home Range in Acres
Ad.	♂	18	March	18	16	8.9
Ad.	♂	36	March	18	15	12.4
Ad.	♂	36	April	10	......	6.9
Ad.	♂	62	April	22	17	3.1
Ad.	♀	20	March	16	......	7.7
Ad.	♀	20	April	23	20	6.5
Ad.	♀	20	May	20	......	7.8
Ad.	♀	53	March	16	15	3.9
Ad.	♀	53	April	20	......	3.6
Juv.	♀	328	May	11	10	5.2

The largest home range of a mature female comprised 7.8 acres, and the smallest, 3.6 acres. The one mature female whose home range in each of the three months is known varied the size of her range within the rather narrow limits of 1.3 acres. Her smallest range comprised 6.5 acres in April; her largest, 7.8 acres in May. The other adult female varied the size of her monthly range by 1.6 acres. Her range equaled 3.6 acres in April and 5.2 acres in May. The five monthly ranges of these females averaged 5.9 acres. One juvenile female that was caught eleven times in May had a calculated home range of 5.2 acres.

POPULATIONS AND CONCENTRATIONS

The maximum population of scorpion mice was on plot A in the last half of March, when there were three mature females and five mature males. This constituted an average population of 0.06 females and 0.10 males per acre, or a total of 0.16 mice of this species per acre. The population on plot B was smaller than that on plot A. There was, on plot B, one mature male and one mature female during the two April periods and the first period of

May. In the last May period there was one adult and one juvenile male. The average population of scorpion mice per acre on plot B comprised 0.04 individuals in every period. This was lower than the average population per acre on plot A in every period except the last one in May, when the averages on the two plots were the same.

The concentration of scorpion mice was not very large, for the average number of individuals of either sex per home-range area never equaled one. The greatest concentration was of mature males in the first half of March on plot A, where there was an average of 0.90 individuals per average home-range area. The greatest concentration of mature females comprised an average of 0.39 individuals per average home-range area in the two April periods and in the first of the May periods on plot A. On plot B the concentration of mature males never exceeded 0.16 individuals per home-range area, and that of adult females never surpassed 0.13 individuals per home-range area.

Cahalane (1941: 62) calculated that there was an average of 2.0 individuals per acre in an area of mesquite and grass in the San Simon Valley, Arizona, in late August, 1933. If the area that he trapped effectively is computed from my home-range data, his average of 2.0 individuals drops to 0.3 individuals per acre.

BREEDING

Sexual activity of the male scorpion mice during the entire trapping time, from early March to late May, was indicated by their enlarged testes. Only one of five males examined in the period of March 11 to 17 appeared not to be in breeding condition. Every other time that a male was examined sexual activity was evident.

Each of the five mature females on the two plots bore one or more litters during the trapping time. Four bore litters about the first of April, and one of these again produced young near the middle of May. A female that was taken only once, on May 10, was pregnant.

The failure to catch any young mice in the early periods of trapping probably indicates that the breeding season did not begin until about the time trapping was begun. In spite of the fact that at least six litters were born on or near the plots, only one immature individual was caught. This possibly resulted from the young dying of neglect while their mothers were in traps.

Bailey (1931: 142) recorded a pregnant female of this species that was taken August 14 at Socorro. It seems probable that the breeding season extends at least into late summer.

Neotoma micropus canescens

The plains wood rat was present on both plots. No systematic trapping of wood rats, however, could be done, because most of the traps were too small to catch these animals. No attempt was made to learn the number of wood rats on plot A, but on plot B a few large traps were set near wood-rat nests and enabled us to mark all or nearly all of the resident wood rats. The wood rats made a nuisance of themselves by overturning the small traps on the plot, so that they were trapped primarily to deter them from disturbing the regular trapping.

POPULATIONS

At least ten wood-rat nests in use were located on plot A, and at least twenty-one on plot B. On the latter plot fifteen wood rats were trapped during April. Seven of these were mature females, six were mature males, and there was one juvenile wood rat of each sex. In May only eleven wood rats were caught on plot B. Only two of these were previously unmarked. The difference in number caught between the two months probably is due to the unsystematic trapping for these animals rather than to any decrease in population density. The fact that the number of active nests exceeded the number of wood rats caught on plot B possibly indicates that one individual utilizes more than one nest. In this connection, one male and one female were caught near two different nests, one male was taken near three different nests, and one female was captured in the vicinity of four different nests. That a wood rat was caught near a nest is not conclusive proof that it was using that nest. The fact that wood-rat nests were twice as numerous on plot B as on plot A undoubtedly indicates a larger population on plot B.

BREEDING

All of the mature wood rats examined on plot B showed evidence of sexual activity. The three females that were caught between April 1 and 7 were suckling young. Each female subsequently examined either appeared to be pregnant or recently to have suckled young. Two suckling females were trapped near Alamogordo on October 30, 1938.

Bailey (1931: 174) recorded a nursing female that was taken at Ancho on September 23 and a half-grown young taken at Tularosa on November 21. He also listed records of summer breeding which, with my data, seem to indicate a rather continuous breeding season from at least early April until October.

MOVEMENTS

No wood rat was caught a sufficient number of times nor in a sufficient number of places to indicate the size of its home range. One adult male was caught at two points about 920 feet apart, but it is impossible to be sure whether this indicated a normal movement within his home range or a shift of range.

SOCIAL RELATIONS

On three occasions a mature male and female were the only wood rats caught in the vicinity of a given nest. On another occasion a juvenile male and female were trapped near the same nest. Near one nest five different individuals, three females and two males, were taken during the two months of trapping. A suckling female was taken there on April 5, but she was not caught again. On May 3 a mature male and female were caught at this nest, both of whom had been previously trapped at different near-by nests. On May 14 and 15 a mature female was caught on the plot for the first time near this same nest, and on May 16 a mature male that previously had been caught at a near-by nest was taken at this nest. These records suggest a considerable amount of overlapping in the home ranges of the wood rats.

Mus musculus

An adult male house mouse was trapped in plot A on April 11. It was caught only once and evidently was only passing through the plot. The enlarged testes indicated that this animal was in breeding condition. No other house mouse was caught on either plot.

HOME RANGES OF MESQUITE ASSOCIATION MAMMALS

The features of mammalian home ranges of most concern are the size, relative stability, and the amount of overlapping of the ranges of the same and different sex and age classes, and of the same and different species. The matter of overlapping ranges, since it throws light on the interrelationships of individual constituents of the population, is discussed in the section on "The Mammalian Population of the Mesquite Association."

SIZE

In size, the home ranges of two species of mesquite association mammals, as shown above, generally are larger than those of other races of the same species studied in the blue-grass, beech-maple, and oak-hickory associations of Michigan. The Chihuahua deer-house (*Peromyscus maniculatus blandus*) ranges more widely than do the prairie deer-mouse (*Peromyscus m. bairdii*) of the blue-grass association and the woodland deer-mouse (*Peromyscus m. gracilis*) of the beech-maple association. The desert wood mouse (*Peromyscus leucopus tornillo*) ranges more widely in the mesquite association than does the individual of another subspecies (*Peromyscus leucopus noveboracensis*) in the oak-hickory association of southern Michigan (Burt, 1940: 26). In these species, therefore, there are marked geographical differences in home-range size probably accounted for by obvious differences in the ecological conditions prevailing in the several associations in which these species were studied.

Most of the small mammals except *Peromyscus* of the mesquite association have no close relatives in the other areas in which home ranges have been studied. Therefore, no comparisons of their ranges can be made with those of others of the same species or even of the same genera. The general statement can be made, however, that characteristic desert mammals, as represented by *Dipodomys ordii, Dipodomys merriami, Onychomys leucogaster,* and *Onychomys torridus* of the mesquite association, usually have larger home ranges than the characteristic small mammals of the eastern deciduous forest and prairie grasslands (Blair, 1940*a*, 1940*b*, 1941*c*, and 1942). A pocket mouse (*Perognathus penicillatus*) proves an exception to this rule, for this species ranges no more widely than some of the forest and grassland mammals. The woodland jumping mouse (*Napaeozapus insignis frutectanus*) also ranges as widely as some of the desert mammals. The exceptionally large range of the woodland jumping mouse possibly is accounted for by its greater morphological adaptation for mobility than is present in other forest inhabitants.

It is extremely interesting and seemingly contradictory to the foregoing observation about the woodland jumping mouse that the presumably most mobile desert mammals, the kangaroo rats, do not have the largest home ranges. The saltatorial kangaroo rats have home ranges of about the same size as the not so well-adapted deer-mice and wood mice. The grasshopper mice and scorpion mice (*Onychomys leucogaster* and *O. torridus*), which appear to be no better adapted for mobility than are the deer-mice and wood mice, have the largest home ranges of the mesquite association mammals. It is possible that, because of their insectivorous habits (Bailey and Sperry, 1929), grasshopper mice and scorpion mice range farther in search of food than do the vegetation eaters.

STABILITY

Each of the mammals of the mesquite association, with the exception of the wood mouse, usually remained in the same general area for a relatively long period of time. The wood mice were definitely nomadic in behavior. One home range was occupied for a short period; then the animal moved on to another. Among the other species each individual that was marked on the plots usually stayed in the same vicinity until the end of trapping, although the limits of its calculated home range varied within usually narrow limits from month to month (Fig. 2). There were, nevertheless, occasional movements of as much as one-third of a mile. In such cases, an animal moved away from an established home range and subsequently came to occupy an entirely different one.

These observations indicate that, in respect to the possible dispersal of hereditary characters through the population, two opposite trends exist. The long-term occupation of limited home ranges by the bulk of the population tends to inhibit the spread of new characters. On the other hand, the occasional movements of some individuals for distances several times the width of a home range facilitates the spread of such characters. Of even more importance in this respect than the occasional movements of mature animals that were observed are the possible movements of young individuals after they leave the nest. No data are yet available, however, on the rate of dispersal of the young of the mammals of the mesquite association.

THE MAMMALIAN POPULATION OF THE MESQUITE ASSOCIATION

The several species of small mammals of the mesquite association have been considered separately in a foregoing section. It is also equally important to consider the total mammalian population of the association as a unit, of which each individual is but one component part.

SIZE OF POPULATION

The average total population of all small mammals, as shown by the trap records, reached a high of 3.64 individuals per acre on plot A in the first half of May. The average for all of the "half-month" periods on this plot comprised 3.24 individuals per acre. A high of 3.19 individuals per acre was reached on plot B in the last half of May. The average for all of the four "half-month" periods on plot B was 2.51 small mammals per acre. These totals, of course, do not include forms that were too large for the traps, such as cottontails, jack rabbits, and wood rats, nor the diurnal ground squirrels.

The total populations of mesquite association mammals seem rather small. Actually, the mammalian population probably was at its lowest point of the year when trapping was begun. A period of sexual inactivity of most of the species ended at about the start of the field work. During that period, when no young were being produced, losses from the population were not balanced by births. The population normally reaches a low just before young animals begin to appear in the spring, and normally reaches a maximum toward the close of breeding in late fall or early winter. No census study of the mesquite association mammals has been made at the season when one would expect to find a peak of abundance. I noted, however, that small mammals seemed much more numerous during extensive trapping in the mesquite association in the fall and in the early winter of 1938 than they were in the spring of 1940.

INTERRELATIONSHIPS OF THE MAMMALS

Since the observational method is not applicable to these small, nocturnal mammals, knowledge of the relationships between individuals and between species is limited to the information supplied by the trap records. Chihuahua deer-mice and four-toed and five-toed kangaroo rats appeared tolerant of their own kind, for their calculated home ranges broadly overlapped the ranges of others of the same species and sex. Breeding female pocket mice (*Perognathus penicillatus*) evidently were intolerant of one another, for their home ranges scarcely overlapped. Of the remaining species, too few individuals were caught to show adequately the amount of overlapping of their home ranges. It is obvious, nevertheless, that the species making up the bulk of the population, the deer-mice and the kangaroo rats, were tolerant of their own kind.

Concerning the relationships between the several species, it is known that the ranges of all overlapped one another indiscriminately. Because of the rather large home ranges of the mesquite association mammals, each individual in its normal travels must, on the average, come in contact with from ten to fifteen other individuals of its own kind and of other species. Nothing is known of what actually happens when an individual encounters another of the same or of a different species. There can be no doubt, however, that some *modus vivendi* has been established whereby numerous individuals of several species occupy the same terrain.

Tappe (1941: 145) believed that other small mammals were scarce in areas inhabited by *Dipodomys heermanni tularensis* because of competition for food from that species of kangaroo rat. No evidence of such severe competition was found among the small mammals of the mesquite association. There undoubtedly is competition in the sense that food eaten by one individual is not available for any other individual. There is an almost unlimited supply of mesquite, however, which, in one form or another, appears to be the principal food of most of the small mammals in this association. It is difficult, in fact, to discover any evidence of competition of a critical nature among these small mammals. The number of holes and tunnels so greatly outnumbers these animals that it is hard to believe that there is any great amount of competition for home sites or refuges, at population levels such as obtained during the time of study.

It is particularly interesting that in this association there are three cases of two rather closely related species of the same genus living side by side and actually occupying overlapping home ranges. The best example is afforded by the kangaroo rats (*Dipodomys ordii* and *Dipodomys merriami*). These two mammals are very similar in size and external appearance, and both have, in general, similar habits. The populations of these species in the mesquite association were both of the same general order of magnitude. Locally, either one might exceed the other in abundance. The grasshopper mouse and scorpion mouse (*Onychomys leucogaster* and *O. torridus*) also occupy overlapping home ranges, with the result that individuals of the two species undoubtedly come in contact with one another. The scorpion mice here are more numerous than the grasshopper mice, which show a preference for sandy soil and sand dunes. The deer-mouse and wood mouse (*Peromyscus maniculatus* and *P. leucopus*) occur together here as in many other parts of their range, although the two are to a considerable extent kept apart by their different ecological preferences. There is no evidence of any critical amount of competition between closely related species for food, home sites, or territory.

SUMMARY

The small mammal population of the mesquite association near Alamogordo, New Mexico, was studied by live trapping from March 4 to May 24, 1940; 105 Chihuahua deer-mice and 419 individuals of ten other species were marked and retrapped many times.

The average size of the home ranges of mature males and females was determined for five species. Some information about home-range size was obtained for two other species, and several facts about the general life history of four other species were learned. Only in the case of the pocket mouse (*Perognathus penicillatus*) did the mature males have significantly larger home ranges than did the females. These pocket mice had the smallest average home ranges of the mesquite association mammals, whereas the scorpion mice had the largest.

Breeding by all of the species of small mammals of the mesquite association was in progress in the spring of 1940. Broad overlapping of home ranges within all sex and age classes occurred in all species except the pocket mouse. In this species the home ranges of mature females did not overlap one another during the breeding season. Occupation of individual home ranges of all species except *Peromyscus leucopus* was relatively permanent, but occasional individuals moved from one home range to another. These movements sometimes were for distances of as much as one-third of a mile.

The four-toed kangaroo rat was the most abundant species on one plot; the five-toed kangaroo rat was the most numerous species on the other. The greatest average number of individuals of all species per acre on either plot was 3.64 in the first half of May. Mature male five-toed kangaroo rats reached the greatest concentration of any sex or age class of any species, for there was an average of 2.43 individuals per average home-range area. A concentration of more than one individual per average home-range area also was found in mature female five-toed kangaroo rats, in mature male and female four-toed kangaroo rats and Chihuahua deer-mice, and in mature male pocket mice (*Perognathus penicillatus*).

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HYBRIDIZATION IN NATURE BETWEEN SPECIES OF CATOSTOMID FISHES

BY

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INTRODUCTION

It may now be regarded as well established that distinct species and even genera of fish hybridize in nature, at times with considerable frequency. The natural crossing of species has a bearing on several lines of biological inquiry—growth, nomenclature, speciation, genetics, and wildlife management—and consequently calls for a thorough analysis. To ensure an intensive and compact treatment we have selected for the present study the hybrids which we have thus far recognized in the Catostomidae, a single family of almost exclusively North American fresh-water fish. Much work has been published[1] in both Eurasia and America on interspecific combinations in the closely related family Cyprinidae, but no hybrids between catostomid fishes (the "suckers") have previously been described.

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[1] Some key references on interspecific cyprinid hybrids are: Heincke (1892), Hubbs and Brown (1929), Schmidt (1930), Hubbs and Schultz (1931), Matsui (1931), Berg

This paper is the product of a division of labor. Carl L. Hubbs made the identifications of the hybrids and is responsible for most of the interpretive treatment. Laura C. Hubbs carried out the almost endless mathematical calculations. Raymond E. Johnson, as research assistant, made nearly all of the counts and measurements (about 2000 on the hybrids and a much larger number on the parental species). We joined in planning and carrying on the details of the investigation and in preparing the report.

Grants from the Horace H. Rackham School of Graduate Studies, of the University of Michigan, made it possible to conduct this analysis and to publish the results. The gifted hand of Grace Eager produced the pictures of the lip structures, which illustrate well the intermediate characters of the sucker hybrids. Leonard P. Schultz collected many of the specimens studied, and collaborated in the first identification of hybrids between *Catostomus macrocheilus* and *C. syncheilus;* he has also loaned us 2 specimens from the National Museum collections. Robert R. Miller carried out the field work on the hybridization between *Catostomus,* species, and *Pantosteus santa-anae* and collected part of the *Catostomus tahoensis* × *Pantosteus lahontan* material. Professor Cecil C. Craig, of the Department of Mathematics in the University of Michigan, derived for us a formula for determining the standard error of the hybrid index (as described below).

MATERIALS AND METHODS

The natural hybrids analyzed in this paper were obtained in the course of the systematic study of North American fresh-water fishes which has been stressed at the University of Michigan Museum of Zoology since 1920. Of the 182 hybrid suckers studied, 76 represent 3 hybrid crosses in the genus *Catostomus* and 105 exemplify 7 hybrid combinations between species of the genera *Catostomus* and *Pantosteus.*

Only a few of the specimens were recognized as hybrids in the field, and even then the collections may be regarded as random, in regard to the numbers of hybrids and of parental species. When the hybrids were identified in the field, as in Sucker Creek, Oregon (where *Catostomus macrocheilus* and *Catostomus syncheilus syncheilus* cross), further collecting was designed to secure series as large as possible, not only of the heterozygotes but also of the parental types. The only trip made for the special purpose of securing additional specimens of hybrid suckers in streams where they are known to occur was to Sespe Creek, California, where *Catostomus,* species, and *Pantosteus santa-anae* cross. Additional material of any available suckers, to augment the hybrid material, was otherwise specifically sought only once—

(1932–33), Schultz and Schaefer (1936), Geyer (1937), Raney (1940*a* and *b*), and Hubbs and Miller (1943). A large number of other references are given in Dean's *Bibliography of Fishes* (1923, 3: 505–7) and in the *Zoological Record.*

from the Colorado River drainage basin of Colorado, where the apparently introduced *Catostomus commersonnii sucklii* was found to be hybridizing with a native sucker, *Pantosteus delphinus delphinus.*

All of the material reported on in this paper is contained in the Museum of Zoology of the University of Michigan (U.M.M.Z.).

The methods of study were those employed in the more precise systematic investigations of the present day. The suspected hybrids were scrutinized for any possible characters of structure, form, or color, by which they might show their relationship to the presumed parental species. Special attention was paid to diagnostic features in the structure of the mouth (Pls. I, II, and VI) : the degree to which the upper lip is flattened out; the tendency for the outer face of the upper lip to be smoother (less papillose) than the inner face, and to be ridged and reflected so as to partly cover the inner surface; the presence or absence of an emargination at the outer lateral angles of the mouth; the depth of the median incision between the lobes of the lower lip, the width of the union in front of the incision, and the number of rows of papillae crossing this isthmus; the width, strength, and shape of the cartilaginous ridges of the jaws, inside the upper and the lower lip; the degree to which the gape is arched, or transverse; and the width and the length of the mouth. The size, shape, and prominence of the snout were also emphasized.

In order to permit statistical analysis of differences in proportions and of differences in the numbers of dorsal fin rays and of scales, all measurements and counts thought to be critical and obtainable with accuracy were made. These determinations were taken according to the methods outlined by Hubbs and Lagler (1941: 12–20). The predorsal length, as here given, is the distance from the tip of the snout to the front of the small, embedded predorsal plate, at a place where, upon slight pressure, the divider point comes to rest. The width of the mouth is the greatest over-all measurement, taken after the lips, if distorted in preservation, have been molded into approximately normal shape. The length of the union of the lower lip is the distance along the mid-line from the front of the cartilaginous ridge of the lower jaw to the point of the V along the extreme posterior edge of the lip, disregarding the shallow groove which extends some distance forward.

Measurements of the appropriate parts of the body were taken with a dial caliper reading to 0.1 mm. and estimated to 0.01 mm. Such refinement has little significance with the larger dimensions, but is important as a source of avoiding errors in measuring small parts. The measurements are calculated as thousandths of the standard length.

Comparable counts and measurements were made on selected material of the parental species as well as on the hybrids. An effort was made to find specimens of the species which were from the same locality as the hybrids and which were about the same size. When such material was not available, examples which were as nearly the same size as could be found were selected from as near by as possible. When they covered a considerable size range the hybrids and parental species were divided into size groups of limited extent, so as to render the proportionate measurements reasonably comparable. These groupings are indicated in the table of proportions for each hybrid combination. The method of relative-growth analysis is more precise, but was not thought practicable for this study because of the small number of hybrids.

The method of counting the pelvic rays needs some comment, as the number of rays in this fin provided a valuable check on the interpretation of 2 types as hybrids. Pelvic ray counts are not generally listed in systematic descriptions of suckers though they have been shown to be of value in this family (Hubbs, 1930: 20–21, 37). The sum of the counts of both fins is the unit of tabulation. A variable sliver-like ray at the outer edge of the base of the first main ray is disregarded. Each of the 2 to several rays that are often approximated or even partly fused is included in the count. Occasional fins that are lacking, rudimentary, defective, or obviously injured are not counted.

In the tabulations and computations all counts for each stated combination were treated as a single statistical array for all the hybrids and for all the specimens studied of each parental species. In contrast, the computations based on measurements were made separately for each size group at each locality.

The means and their standard errors are given. The standard errors of the means (S.E.) were computed according to the formula

$$(1)\quad \text{S.E.} = \frac{\sigma_V}{\sqrt{N}}$$

The standard deviation (σ_V) of the noncontinuous variates was obtained according to the commonly used formula

$$(2)\quad \sigma_V = \sqrt{\frac{\Sigma d^2}{N-1}}$$

The coefficient of variability is

$$(3)\quad V = \frac{100\,\sigma_V}{M}$$

The counts are all tabulated according to the hybrid combinations (Tables XXXII to XXXVII). Because of the variations with age and locality, no simple frequency tabulation of the proportionate measurements is warranted. We can, however, obtain figures (hybrid indices) which portray the relation of each measurement in each hybrid to the mean value for each parental species of a comparable size group from the same or an approximate locality. We can then combine these indices in any way that is desirable.

Character Index

Owing to the approximation of their characters and to the considerable individual variability which they display in each respect, it is difficult to identify suckers and their hybrids on the basis of single traits. The use of combinations of characters leads to more certain identifications. For this reason we have found character indices of particular value in the present study.

The term "character index" was proposed by Hubbs and Whitlock (1929: 470), who found the need for a simple mathematical expression of several differential features. Following this introduction, character indices have been successfully applied in fish systematics for the separation of closely allied forms by Koelz (1929: 426), Schultz and Welander (1934: 6), Hubbs and Cannon (1935: 27), Schultz and Thompson (1936: 74–75), Hubbs (1936: 189–93 and 249–50), Hubbs and Kuhne (1937: 13–14), Schultz (1937: 19–20), Schultz and Reid (1937: 211–12), and in a few other works. Schultz and Schaefer (1936: 5–8) first applied a character index in the determination of fish hybrids. Hartweg (1938: 3–5) and Woodbury and Woodbury (1942: 138), respectively, applied the method in chelonian and ophidian systematics.

The character index, under the name of "general index" or "aggregate difference," has also been introduced in botany (Anderson and Whitaker, 1934: 35–38, Fig. 4; Anderson and Abbe, 1934: 43–49, Fig. 1; Stebbing, 1942: 228–30). The method of computation of the botanist's index (taking the square root of the sums of the squares of the individual differences) has not been employed in ichthyology, but the scope and significance of their index, as stated, is the same as that of the ichthyologist's character index. It is of some interest that methods for summarizing characters into a single index figure were independently proposed by botanists and ichthyologists.

The use of character indices and of other statistical combinations of characters, such as the physical anthropologist's "coefficient of racial likeness," has lately been severely criticized by Ginsburg (1939). We see no reason, however, to doubt the value of the method. Proportions and ratios, long used in systematic ichthyology, are simple character indices—these, no one queries. It is the somewhat more complicated formulae that, on first attention, may appear to be incongruous or to be the mere juggling of figures. No arithmetic combination of characters, however, can bring out differences which do not exist, so long as we apply the identical character-index formula to both types being compared. When the data are limited a source of error may lie in the selection of character combinations that happen to yield results in line with expectancy.

Contrary to Ginsburg's claim, character indices may measure not only the significance of differences but also degrees of divergence. Very few, if any, kinds of animals differ in a single character. The forms are separated by combinations of characters. Races and subspecies, as well as "good species," tend to exhibit what Goldschmidt (1940) has called "different reaction systems" (Hubbs, 1941*c*: 276). Since the types are inherently characterized by combinations of characters, the validity and the amount of their differentiation might often be better indicated by indices which measure several features than by simple counts or measurements of single traits. Only the real difference between 2 populations is shown by a character index which, on the basis of adequate samples, largely or wholly eliminates the overlap in frequency distribution that is evident when one considers separately the traits which were combined to form the index. Forms which differ consistently in an ensemble of characters must have attained full specific distinction.

The computation of a character index somewhat resembles the action of the mind in the impressionistic identification of kinds of organisms. The experienced mind which with little error can distinguish Japanese from Chinese men synthesizes a large number of more or less unrelated features, any one of which if measured and analyzed would show a great overlap in frequency distribution.

One main advantage of a character index—and this advantage tends to accrue, the larger the number of traits combined—lies in the fact that a character aberrant enough to cause one form to resemble another with which it is being compared tends to be offset by other characters which will probably not vary in the same direction. The character index gives joint expression to a number of traits, minimizing the importance of extreme fluctuations in single features.

The present work provides examples of the utility of a character index in sharpening the expression of the difference between populations; in our case between a hybrid and each of its parental species. Thus, a character index (Table V; Fig. 1) based on the scale and ray counts of *Catostomus macrocheilus,* hybrids, and *C. syncheilus* shows the relations between the hybrid and its parent species much more emphatically than does any of the sets of counts considered alone (Tables XXXII–XXXVII; Fig. 7). Again, subtracting certain scale counts that are low in one type from those that are high in the same form, gives us an especially sharp and valuable indication (Table XVIII; Fig. 5) of the difference between certain hybrids and their parental species. The difference thus shown is a true one, reflecting only what exists in the organisms—in no sense an artificial concoction.

Some doubts may arise as to the statistical treatment of character indices, but we have been informed by statisticians that such values as standard deviation, standard error, and the like may legitimately be computed for an array of the indices, provided that each of the combined characters presents in itself an approximately normal frequency distribution. This proviso will ordinarily be met.

Hybrid Index

The hybrid index, a special type of character index, is designed as a simple expression of the relationship of the traits of the hybrid to those of the parental species. It may be obtained separately for each character of each hybrid specimen, and the individual indices may then be tabulated for all the hybrids to show, for example, how the average number of dorsal rays or the average width of the mouth of the hybrids, as a group, compares with the mean value for each parental form (Table XXXVIII). Also, the separate indices for all traits in any given hybrid specimen may be averaged so as to indicate how the individual, in the ensemble of its characters, compares with each parental type (Table XXXIX; Fig. 8).

Anderson (1936) proposed such an index for the analysis of plant hybrids, and his method has been followed by other plant geneticists and systematists (cited by Riley, 1939). This method is not regarded as adequate for the analysis of fish hybrids. It assigns the value 0 to the measurement or count of one species, 2 to that of the other parental form, and 1 to that of the hybrids, if they are intermediate. But there are all degrees of intermediacy, and every gradation from the one parental type to the other.

A somewhat similar method for computing a hybrid index has just been used by Ripley (1942: 57–58, Fig. 1), in an analysis of hybridization between species of birds. By judging the differential degree of resemblance Ripley assigned scores for each character, on a scale grading from 0 for one parental species to 10 for the other, and then averaged these scores for each hybrid specimen, so as to provide a numerical expression of its similarity to each species.

The hybrid index as here used was introduced by Hubbs and Kuronuma (1942: 291). In any comparison each individual or average index is expressed as a figure on a scale of 100, in which the average value for the one parental type is set at 0, and that for the other parental type at 100. With V_H representing the value of the character of the hybrid, M_1 the mean value for parent, 1, and M_2 the mean for parent 2, the formula is, therefore:

$$(4)\ \text{Position of the hybrid (P)} = \frac{V_H - M_1}{M_2 - M_1}$$

$$(5)\ \text{Hybrid index (I)} = 100\,P$$

In the present paper we have applied M_1 to the parental species which seems to be the more primitive. This is the coarse-scaled, short-nosed species in the *Catostomus* × *Catostomus* crosses, and the *Catostomus* species in the *Catostomus* × *Pantosteus* hybrids. It is recommended that such practice be followed whenever a decision as to differential primitiveness is feasible.

It will be noted that a hybrid index of 50 denotes exact intermediacy between the parental types. A summary index of 25 in a *Catostomus* × *Pantosteus* hybrid would indicate a correspondence 3 times closer to the *Catostomus* parent than to the *Pantosteus.*

The hybrid index is of particular service in testing the assumption of hybridity and the mode of inheritance. If the assumed hybrids are merely selected specimens of the 2 species, or if characters show strict Mendelian segregation, the individual indices when combined would show a bimodal frequency distribution with modes close to 0 and to 100 on the scale mentioned above. A unimodal curve with the peak near 50 would confirm the interpretation of the aberrant specimens as hybrids, and would indicate a multiple-factor type of inheritance for systematic characters (or—as a generally improbable contingency—merely a lack of dominance). Should the values for 2 or more unrelated traits in a given set of hybrids yield such unimodal curves, intervening between those for the parental species, the assumption of hybridity would appear to be well established.

An average index of about 50 could be obtained by mixing typical specimens of each form, or through Mendelian segregation, which would cause the hybrids to resemble one parental species in some respects and the other parental type in other characters. But in either case the frequency distribution would be bimodal. The fact that the summary indices for all characters for each individual tend to average near 50 might also result from a mixture of specimens of the 2 types or from genetic segregation. To clarify the situation, therefore, the indices need be summarized for single characters and for all characters, as well as for individual fish.

The hybrid index is computed directly for hybrid populations by substituting the mean value of the hybrids (M_H) for V_H in formula (4).

When the number of hybrids and of each parental species approximates or exceeds 30, when the frequency distribution is approximately normal (as it usually is), and when the dispersion of the difference between the values for the 2 species is relatively small as compared with the difference between the average for the parental forms, it is possible to make an approximate computation of the standard error of the hybrid index ($S.E._I$) as follows:

$$(6)\quad S.E._I = 100\sqrt{\frac{\dfrac{\sigma_1^2+\sigma_2^2}{(M_2-M_1)^2}\left[P^2-2P\dfrac{\sigma_1^2}{\sigma_1^2+\sigma_2^2}+\dfrac{\sigma_H^2+\sigma_1^2}{\sigma_1^2+\sigma_2^2}\right]}{N_H}}$$

In this formula $\sigma =$ standard deviation of the mean (M); the subscripts refer to parent 1, parent 2, and the hybrid; P is the position of the hybrid (formula 4); and N_H is the number of hybrids.

CRITIQUE ON SUPPOSED HYBRIDIZATION BETWEEN SPECIES OF *MEGASTOMATOBUS, ICTIOBUS, CARPIODES,* AND *MOXOSTOMA*

Before presenting our original data we consider the only previously published statement on hybridization in the Catostomidae. Thompson (1935: 492–93) wrote:

> While it has not been proved that the so-called "mongrel" buffalo, *Ictiobus urus,* is really a hybrid between the other two native species, there is a considerable body of circumstantial evidence to indicate that such is the case. Some of the difficulties of identification in the genus *Carpiodes* and in the genus *Moxostoma* may be attributed to occasional hybridization within these genera.

Issue may be taken with these statements. Difficulty of identification in the genera *Ictiobus, Carpiodes,* and *Moxostoma* is admitted, but appears to be due not to hybridization but rather to 2 other factors: (1) a tendency for the species to be characterized by a combination of traits, none of which by itself may be invariably distinctive; (2) a common failure to recognize or to appreciate all of these characters. The rather clear diagnoses of the species of *Ictiobus* (including *Megastomatobus*) and of *Carpiodes*, given by Forbes and Richardson (1909, and 1920: 68–80) were supplemented by the study of Hubbs (1930: 8–16). Most of the characters of the Mississippi and Great Lakes species of *Moxostoma* are rather weakly distinctive when considered individually, but are adequate for identification when taken in combination (Hubbs, 1930: 16–32). When all the characters presented by these authors are taken into account and the specimens are subjected to intensive study, doubt seldom need arise concerning the specific identity of any of the suckers of the Mississippi Valley and Great Lakes region (with the probable exception of 3 pairs of species, discussed below).

The suggestion that *Ictiobus urus* (= *I. niger*) may represent hybrids between *Ictiobus* (= *Megastomatobus*) *cyprinella* and *Ictiobus bubalus* finds very little if any confirmation. It is true that some of the characters of *I. niger* are intermediate between those of *M. cyprinella* and those of *I. bubalus*, but other features of *I. niger* are not intermediate. Apparently having in mind the idea of hybrid origin expressed in the fisherman's vernacular name, Forbes and Richardson held:

> The mongrel buffalo [*I. niger*] appears to vary somewhat more than either *I. cyprinella* or *bubalus*, but we have met with no cases which appear to show intergradation with either. This species seems to be always distinguishable from the former by its much smaller and less oblique mouth, the upper lip falling far below the level of the lower margin of the orbit, and by the coarsely striate lower lips; from the latter by the more elongate and less compressed body, and by the broad rounding of the frontal region and of the back in front of the dorsal fin.

In the anatomical characters treated as of generic implication (Hubbs, 1930: 8), *Ictiobus niger* agrees with *I. bubalus*, and both these species therefore contrast sharply with *Megastomatobus* (the only partial exception to this statement involves mouth structures). Krumholz (1943: 37–38, Pl. 1) finds not only that *I. niger* is sharply distinctive in the characters of the Weberian ossicles, particularly in the sculpturing of the tripus, but also that it is not intermediate in these features. *Ictiobus niger*, therefore, does not conform to the virtually universal rule (Hubbs, 1940: 205–9) that interspecific hybrids have uniformly intermediate characters. In general, *I. niger* is associated with the 2 other species, but it occurs alone in parts of its range (Hubbs, 1930: 11; Hubbs and Lagler, 1941: 41), therefore, in places where hybridization would not be expected.

The theory of hybridization between *Megastomatobus cyprinella* and *Ictiobus bubalus*, at least as a common phenomenon, seems ruled out by the evidence. It would be difficult to prove, however, that hybridization does not take place between some of the more closely related species in the Ictiobinae. Because of extensive variation in each species of the 3 following combinations, it would be hazardous to identify hybrids between: *Ictiobus* *niger* and *I. bubalus; Carpiodes forbesi* and *C. cyprinus; Carpiodes carpio* and *C. velifer.*

These pairs are trenchantly distinguished from other pairs, but the 2 members of each unit have very similar characters (Hubbs, 1930: 8–18). The distinctive features—parallel within each of the 3 pairs—mainly involve depth and compression of body, the expanse of the fins (particularly the dorsal), and the size of the eye. The first-named member of each pair is a slenderer, thicker fish than its cognate and has a lower dorsal fin, a larger mouth, and a smaller eye. These characters, however, exhibit so much individual and age variation that it is extremely hard at times to separate the related forms. It seems probable that the difficulties encountered in separating *I. niger* from *I. bubalus,* or *C. forbesi* from *C. cyprinus,* or *C. carpio* from *C. velifer* are due to an inadequate comprehension of these variations, to the closeness of the characteristics, or to insufficient analytical study. It cannot be regarded as demonstrated, however, that hybridization or intergradation does not occur between the 2 cognate species in each of the 3 pairs.

Until the material was re-examined in the preparation of this paper, we thought that we had sufficient evidence to indicate the occasional hybridization in nature between species of *Moxostoma.* Mention may be made of the 2 specimens formerly treated as hybrids in this genus, because they serve as examples of pitfalls into which one may stumble unless the identification of natural hybrids be made in a critical spirit and with full knowledge of the facts involved.

One of the 2 specimens, an adult from the Muskegon River, Michigan, showed, when fresh, colors intermediate between those of *Moxostoma rubreques* and *M. erythrurum* (with which it was collected). Some of the counts and measurements intervened between those of the supposed parental species; other characters were similar to those of each species. A hybrid index of 47 indicated only a 3 per cent divergence from exact intermediacy. Confirmation of the hybrid interpretation seemed to come from a special measure of the degree of falcation or roundness of the dorsal fin, obtained by dividing the length of the eighth dorsal ray into that of the first principal ray, with results as follows:

In 9 comparable specimens of *M. rubreques*	1.27 to 1.37	(average, 1.32)
In the 1 specimen misidentified as a hybrid	1.47	
In 8 comparable specimens of *M. erythrurum*	1.40 to 1.67	(average, 1.58)

On final recheck, however, it was thought possible that this specimen might be an example of *Placopharynx carinatus,* from beyond its supposed limits of distribution. An examination of the molar pharyngeal teeth verified this suspicion. It was just a coincidence that in certain respects this specimen of *Placopharynx* proved intermediate between 2 species of *Moxostoma.*

Another specimen of *Moxostoma,* taken with *M. aureolum* and *M. anisurum* in the Mississippi River, Wisconsin, was long interpreted as a hybrid between those species. The feature that first attracted attention was the intermediate form of the lips (Pl. II, Figs. 3*a*–*c*). The fish was deeper and heavier-set than the usual (though not the extreme) *aureolum,* even deeper than the average *anisurum.* In some measurements it proved intermediate, in others beyond the range connecting the averages for the 2 species. The hybrid index was computed as 46, strongly verifying the assumption of hybrid origin. Yet other characters, such as the number of dorsal rays (12), the length of the dorsal base (about two-thirds distance forward to occiput), the length of the depressed dorsal (about equal to the dorsal–occiput interspace), the sharp point and moderate falcation of the dorsal fin, and the dark bases of the scales, were wholly typical of *aureolum,* not approaching the expression of these characters in *anisurum* (Hubbs, 1930: 16–32). Such is not the genetic behavior of hybrids between systematic forms. Judgment was accordingly revised; the specimen was reidentified as an aberrant example of *M. aureolum* in which, for some reason, the juvenile V-shaped form of the lower lip was retained in the half-grown (the size at which the lip becomes truncate varies greatly).

We are therefore able to present evidence for interspecific catostomid hybrids only within the genus *Catostomus* and between that genus and *Pantosteus.*

IDENTIFICATION OF NATURAL HYBRIDS BETWEEN SPECIES OF CATOSTOMIDAE

A skeptical attitude should obviously be maintained toward the identification as interspecific hybrids of any fish specimens collected in nature. Since ordinarily the evidence for such an interpretation will remain circumstantial, every effort should be made to exclude other plausible explanations. Needless to say, the trustworthy identification of natural hybrids calls for a thorough knowledge of the systematics of the group.

We have striven to avoid errors of interpretation in the identification of the catostomid hybrids. The supposed hybrid specimens have been thoroughly examined, and many diagnostic counts and measurements taken for detailed comparisons. Their characters have been compared with those of all species which were taken at the same place, or which might be expected to occur there. Due consideration has been given the possibility that the aberrant features of the presumed hybrids were caused by modification or mutation, for it is well known that the characters of one species may be altered to approach or even to correspond with those of a related species (witness the recent study on *Catostomus* by Hubbs, 1941*a*). The heredity that would have been involved in the production of the sucker specimens interpreted as hybrids has been compared constantly with the known genetics of experimentally induced hybrids in other families of fishes.

Circumstantial evidence of a varied nature has led us to diagnose certain suckers as hybrids. It was first determined that an apparent hybrid had characters that exclude it from the limits of normal variation of any species which does or may occur in the same region, or which might have wandered or have been transplanted therein. It was seen not to have the earmarks of a mutant sport. It was determined that all the appreciable characters of the ''hybrid'' were plausibly to be interpreted as the genetic result of the crossing of 2 species which are known to live in the same water or which can reasonably be assumed to occur there. It was thought legitimate to expect that hybrid suckers would follow the general rule (Hubbs, 1940: 205–9) that interspecific hybrids tend to be intermediate between the parental species in all systematic characters.

Careful consideration was given to the possibility that the presumed hybrids might represent hitherto undescribed species. The argument against such an interpretation usually ran as follows: the aberrant fish are too few to represent an established species, in regions as well worked as those from which the hybrids came, and they are too uniformly intermediate between the presumed parental species, both of which occur in the same area. In various features of distribution and ecology as well as in characters, they are most plausibly to be interpreted as hybrids and correspond with authenticated species-crosses in other groups.

The intermediate characters serve most strikingly as evidence for hybridity, when the supposed parental species are separated generically. Throughout many of the waters of western North America the suckers are referable to 2 types, known as *Catostomus* and *Pantosteus*. Ordinarily, these types remain sharply distinct, particularly in the structure of the mouth (Pl. I). Only occasional specimens (those interpreted as hybrids) are sufficiently intermediate in mouth characters to break down the distinction.

The same specimens that show intervening features in one set of characters, such as mouth structure, are also intermediate in various other features of form, counts, and measurements. Nowhere have breeding populations of the intermediate types been collected. They do not have the attributes of species. The only interpretation that fits the facts is that these aberrant specimens are the product of the natural crossing of distinct species.

Most of the hybrids were accorded the final identification on first examination, so striking was the intermediacy in appearance. A shift in opinion on further analytical study was made only for 2 specimens of the *Moxostoma* group (described above) and for a few young of the *Catostomus macrocheilus* × *Catostomus syncheilus* series.

Perhaps the most serious initial doubt was involved in the identification of certain suckers from the Colorado River system of Colorado as hybrids between *Catostomus commersonnii sucklii* and *Pantosteus d. delphinus*. In the first set obtained, the hybrids were small; and no coarse-scaled *Catostomus* of any kind was known from the upper waters of the Colorado system. The occurrence there of such a species was called for, however, to explain the source of the characters of the hybrids, of which *Pantosteus delphinus* (taken there) was obviously one parental species. The second parent was identified as *Catostomus commersonnii sucklii* on the seemingly hazardous assumption that this species of the Mississippi Valley had been transplanted (as bait?) across the Continental Divide. A suggestion that this might have happened came from the inclusion of 2 Mississippian minnow species (*Semotilus a. atromaculatus* and *Pimephales p. promelas*) in the small series containing the unexpected hybrid. This collection was seined in the headwaters of the Colorado River at Hot Sulphur Springs, Colorado. We have been informed that this is a favorite fishing spot for residents of Denver, who may very well have carried live bait minnows across the divide; and in 1942 we noticed that this stretch of water was being heavily fished by Colorado residents (mostly fly-fishermen, however). After the Hot Sulphur Springs collection was studied, material of *Catostomus commersonnii sucklii,* as well as of *Pantosteus d. delphinus* and of 2 additional hybrids between these species, was taken in the Colorado River system of southwestern Colorado. The original diagnosis was thus upheld. Furthermore, the first *Catostomus catostomus griseus* to be recorded from the Colorado River drainage was included, to confirm the view that fishes native to the eastern drainage have somehow gotten over the Continental Divide. The accidental transfer of another bait fish across the Continental Divide has recently been reported by Simon (1942: 7, 17), who wrote that *Platygobio gracilis* has thus become established in the Snake River system. That *Catostomus commersonnii sucklii* and *Catostomus catostomus griseus* (and *Semotilus* and *Pimephales*) are not native in the Colorado River system is indicated by the reports of Jordan (1891), Ellis (1914), and all other ichthyologists who have worked in the Rocky Mountain region.

Mr. C. N. Feast and John D. Hart of the Colorado Fish and Game Commission have just told us that they too think that Platte River suckers have been introduced into the Colorado River system. Suckers first appeared in the South Mesa Lake country in the Colorado headwaters about 1926, and it is thought that they were carried over by bait fishermen, perhaps by Japanese farm laborers, who were brought in about that time. Since then, suckers, thought not to be of the native species, have multiplied so fast in the Colorado River waters as to become a problem.

With the exception of the above-mentioned collection from Hot Sulphur Springs, both presumed parental species were taken with the hybrids, or, in the case of small collections, at a locality near by. When all the available evidence is considered, it seems obvious that both parental kinds live wherever hybrid suckers occur.

One of the strongest lines of evidence in favor of our interpretation of certain suckers as hybrids is the circumstance that these specimens are intermediate between the specific parental types occurring in the same region. Thus, the specimens regarded as hybrids between *Catostomus* and *Pantosteus* are not only intermediate between the 2 genera but also show their affinity to the particular species of these genera with which they were taken. The evidence for this statement can be adduced from the detailed accounts for each combination and is well shown in the comparative drawings of lip structure (Pl. I).

Unfortunately, no opportunity has arisen in the present study to confirm by breeding experiments or by cytological observations the strong circumstantial evidence that the supposed hybrid suckers are really the result of interspecific crossing. However, all the available systematic and natural history information checks with the data on hybrid combinations which have been verified by controlled matings; by observations of the 2 supposed parental species spawning together in nature; by evidence of hybrid vigor in growth, pigmentation, etc.; and by peculiarities in the cytological structure and in the behavior of the gametes. Such confirmation has been provided in some of our other studies (as those of Hubbs and Hubbs, 1932–33).

HYBRIDS BETWEEN SPECIES OF *CATOSTOMUS*

Except for the combination of *Catostomus macrocheilus* with 2 subspecies of *Catostomus syncheilus*, hybrids within the genus *Catostomus* prove to be extremely rare. This rarity of hybrids is due in part only to the circumstance that a single species of *Catostomus* occurs over much of the range of the genus; there are large areas in which 2 species live together. The 5 known *Catostomus* × *Catostomus* specimens, other than those between the 2 Columbia River basin species named above, are referable to the cross:

1. *Catostomus commersonnii sucklii* × *Catostomus catostomus griseus*

(Pl. II, Fig. 2*b*; Pl. III, Fig. 2)

U.M.M.Z.[1] No. 105634: 1 yearling 68 mm. in standard length, collected by W. Petravicz and Middendorf in the North Fork of South Platte River near Pine, Jefferson County, Colorado, at an altitude of 6900 feet; July 20, 1938.

U.M.M.Z. No. 132230: 1 half-grown 151 mm. long, seined by Carl L. Hubbs and family in Laramie River (tributary of North Platte River), east of Bosler, Albany County, Wyoming, at an altitude of about 7000 feet; September 17, 1934.

U.M.M.Z. No. 135190: 2 young and 1 yearling, 33, 44, and 101 mm. long, taken by Raymond E. Johnson and Paul Romberg in North Platte River near Henry, Scotts Bluff County, Nebraska; July 23, 1940.

No specimens of *Catostomus commersonnii sucklii* were included in the small collection containing hybrid specimen, U.M.M.Z. No. 105634, but examples of this parental species were secured by the same collectors near by. No other species of *Catostomus* occur in the region. After re-examining their material we can affirm that Evermann and Cox (1896: 391) erred in referring the fine-scaled suckers of the Platte River system to 2 full species, *C. griseus* and *C. catostomus*. Our studies have led to the conclusion that *griseus* is the southern, montane representative of *C. catostomus* and that it differs from the typical subspecies most noticeably in the greater height and width of the snout, which is less pointed in either dorsal or lateral aspect: except in fish less than 100 mm. long the least distance between the nostrils is more instead of less than two-thirds the postorbital length of the head (Table III).

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[1] U.M.M.Z. refers to the catalogue of fishes in the University of Michigan Museum of Zoology.

There are some indications that *C. c. griseus* may include 2 recognizable races, a fine-scaled mountain form and a relatively coarse-scaled one on the Plains. Unless this be true one might conclude that there is, or has been, considerable hybridization on the Plains between *griseus* and the very coarse-scaled Plains white sucker, *C. commersonnii sucklii* (see Evermann and Cox, 1896: 392–94). In other characters, however, the Plains specimens of *griseus* with as few as 83 to 90 scales in the lateral line (including those so identified by Evermann and Cox) show no distinctive approach toward *sucklii;* we regard them as direct variants of *griseus,* rather than as products of recent or past hybridization. Southern, lowland, and other warm-water races in fishes commonly tend to show a reduced number of scales and other segmental structures (Hubbs, 1918, 1921, 1925, 1926, 1927, 1934, 1940, 1941*a* and *b;* Hubbs and Kuhne, 1937).

TABLE I

Relative Number of Hybrids, *Catostomus commersonnii sucklii* × *Catostomus catostomus griseus,* and of the Parental Kinds

Specimens in University of Michigan Museum of Zoology	*Catostomus commersonnii sucklii*	Hybrids	*Catostomus catostomus griseus*
In collection containing hybrid			
U.M.M.Z. No. 105634	0	1	23
U.M.M.Z. No. 132230	12	1	12
U.M.M.Z. No. 135190	157	3	6
Total	169	5	41
From the Platte River system	1250*	5	692

* Some of these specimens came from localities east of the known range of *C. c. griseus.*

The 5 hybrids between *Catostomus commersonnii sucklii* and *Catostomus catostomus griseus* are essentially intermediate in their ensemble of characters, though in the combined hybrid index (Table XXXIX) one of the specimens closely approaches *sucklii* and one is somewhat more like *griseus* than an exact intermediate. Taking all characters into account, however, it is believed that all 5 specimens are correctly diagnosed as hybrids.

In number of dorsal rays (Table XXXII) the specimens average intermediate, but on this basis alone could not be checked as hybrids since the modal number of rays is only 1 different in the 2 kinds (11 in *sucklii* and 10 in *griseus*). The scale counts (Tables XXXIII to XXXVII) are more diagnostic. The number of transverse rows along the position of the lateral

TABLE II

PROPORTIONATE MEASUREMENTS OF HYBRIDS BETWEEN *Catostomus commersonnii sucklii* AND *Catostomus catostomus griseus*, AND OF THE PARENTAL SPECIES

The measurements are expressed as thousandths of the standard length. There are given for each item the range of the measurements; the average, in parentheses; and, as a subscript, the number of specimens.

	North Platte River, Wyoming and Nebraska			South Platte River, Colorado
Size group, mm.				
C. commersonnii sucklii	32–57 (44)$_{10}$	66–125 (98)$_{7}$	126–182 (148)$_{8}$	77–131 (103)$_{4}$
Hybrids	33–44 (39)$_{2}$	…… (101)$_{1}$	…… (151)$_{1}$	…… (68)$_{1}$
C. catostomus griseus	32–57 (43)$_{9}$	64–113 (93)$_{9}$	127–173 (144)$_{7}$	68–156 (90)$_{10}$
Body depth				
C. commersonnii sucklii	197–215 (204)	193–215 (205)	187–211 (196)	208–225 (216)
Hybrids	203–214 (209)	…… (193)	…… (179)	…… (208)
C. catostomus griseus	167–219 (193)	174–207 (192)	176–200 (190)	172–191 (179)
Caudal peduncle, depth				
C. commersonnii sucklii	82–97 (93)	95–108 (98)	83–96 (89)	92–101 (97)
Hybrids	92–98 (95)	…… (98)	…… (86)	…… (105)
C. catostomus griseus	84–102 (91)	87–107 (96)	75–90 (86)	88–101 (95)
Head length				
C. commersonnii sucklii	279–305 (293)	264–293 (283)	274–297 (283)	254–274 (265)
Hybrids	277–291 (284)	…… (254)	…… (272)	…… (292)
C. catostomus griseus	267–296 (286)	235–272 (258)	227–256 (242)	230–270 (257)
Head depth				
C. commersonnii sucklii	171–182 (178)	155–181 (173)	166–181 (174)	165–174 (171)
Hybrids	157–170 (163)	…… (145)	…… (162)	…… (168)
C. catostomus griseus	153–164 (159)	137–160 (147)	129–148 (135)	129–160 (146)
Head width				
C. commersonnii sucklii	163–175 (168)	155–176 (166)	163–176 (168)	160–177 (167)
Hybrids	152–152 (152)	…… (157)	…… (156)	…… (163)
C. catostomus griseus	142–168 (160)	150–170 (159)	147–162 (153)	146–161 (153)
Snout length				
C. commersonnii sucklii	103–125 (114)	116–136 (126)	121–143 (131)	113–128 (124)
Hybrids	108–109 (109)	…… (123)	…… (126)	…… (131)
C. catostomus griseus	108–122 (116)	108–130 (118)	108–120 (115)	104–124 (112)

TABLE II—(*Cont.*)

	North Platte River, Wyoming and Nebraska			South Platte River, Colorado
Mouth width				
C. commersonnii sucklii	72–85 (77)	67–90 (77)	73–82 (76)	74–90 (83)
Hybrids	82–85 (83)	…… (95)	…… (74)	…… (79)
C. catostomus griseus	82–95 (87)	80–101 (89)	83–98 (92)	78–102 (86)
Cartilaginous ridge, width				
C. commersonnii sucklii	40–48 (42)	39–53 (45)	40–47 (44)	39–44 (41)
Hybrids	45–49 (47)	…… (56)	…… (43)	…… (48)
C. catostomus griseus	49–61 (53)	41–62 (52)	41–53 (47)	49–61 (54)
Mouth length				
C. commersonnii sucklii	51–63 (56)	52–63 (56)	55–63 (58)	53–66 (58)
Hybrids	62–63 (63)	…… (64)	…… (58)	…… (63)
C. catostomus griseus	55–69 (62)	57–71 (63)	60–70 (65)	56–68 (63)
Lower lip, union				
C. commersonnii sucklii	7–18 (11)	13–18 (15)	12–14 (13)	12–13 (13)
Hybrids	9–9 (9)	…… (16)	…… (13)	…… (16)
C. catostomus griseus	11–13 (12)	12–18 (14)	9–17 (14)	13–19 (15)
Dorsal fin to occiput, distance				
C. commersonnii sucklii	240–262 (253)	254–278 (265)	254–284 (273)	262–289 (277)
Hybrids	239–248 (243)	…… (270)	…… (274)	…… (253)
C. catostomus griseus	220–261 (240)	250–299 (270)	284–296 (291)	255–305 (279)
Dorsal fin base				
C. commersonnii sucklii	153–179 (170)	156–178 (166)	153–173 (164)	143–170 (157)
Hybrids	158–161 (159)	…… (153)	…… (148)	…… (184)
C. catostomus griseus	146–172 (159)	133–152 (144)	125–154 (140)	129–155 (141)
Depressed dorsal fin, length				
C. commersonni sucklii	241–271 (255)	252–267 (257)	241–264 (253)	224–269 (246)
Hybrids	235–246 (241)	…… (238)	…… (231)	…… (269)
C. catostomus griseus	234–253 (241)	210–239 (224)	190–222 (211)	199–237 (220)

line is the most critical count, for in this character the 2 parental species are separated by a wide gap, and 4 of the 5 hybrids show no overlap on the range of variation of either.

In the proportionate measurements (Table II) the difference between the parental species is generally slight, and many of the variations overlap widely. It is not surprising, therefore, that in these respects the hybrids show little clear-cut evidence of their mixed parentage. As a whole there is an average tendency toward intermediate proportions. The intermediacy is perhaps best shown, by 4 of the 5 hybrids, in the proportionate length of the depressed dorsal fin.

Better evidence for the hybridity of the 5 specimens comes from other characters (Pl. III, Figs. 1–3). Compared with *C. commersonnii sucklii*, *C. catostomus griseus* has more even contours, less gibbous in the anterior trunk region, less constricted toward the caudal peduncles; the hybrids are in between. The snout of *griseus* is flatter on top than that of *sucklii* and is less decurved in dorsal outline, and it has a much more swollen tip which projects much farther beyond the strictly inferior, rather than somewhat oblique, mouth; in these respects, too, the hybrids are visibly intermediate. In specimens about 100 mm. in standard length the snout of *griseus* is distinctly longer than the postorbital, that of the hybrid is about as long, and that of *sucklii* is definitely shorter than the postorbital. In smaller specimens the snout is relatively shorter in both species and in the hybrids, but the relative distinction holds. Similar intermediate hybrid relations hold for the width of the snout, as measured by the internarial space (Table III).

TABLE III

PROPORTIONATE WIDTH OF SNOUT ON HYBRIDS BETWEEN *Catostomus commersonnii sucklii* AND *Catostomus catostomus griseus*, AND OF THE PARENTAL SPECIES*

Expressed in terms of the internarial distance (between median rims of anterior nostrils) stepped into postorbital length of head (distance from posteroventral rim of eye to free edge of subopercular membrane). Average values, with number of specimens in parentheses.

	Internarial Space in Postorbital Length			
Length groups of specimens	To 50 mm.	50–100 mm.	100–150 mm.	Over 150 mm.
Catostomus commersonnii sucklii	2.0 (12)	1.9 (17)	1.9 (21)	1.8 (8)
Hybrids	1.9 (2)	1.7 (1)	1.6 (1)	1.7 (1)
Catostomus catostomus griseus	1.8 (17)	1.6 (18)	1.4 (19)	1.4 (11)

* These data were obtained too late to be used in the subsequent computations of hybrid intermediacy.

The hybrids are transitional again in the markedly different mouth structures (Pl. II, Figs 2*a–c*). Compared with *sucklii, griseus* has the whole mouth more horizontal, flatter, and more expansive. Both lobes of the lower lip are fuller, therefore with a longer separating incision. The papillae of the lower lip are coarser. The upper lip of *griseus* (approaching that of *Pantosteus*) is somewhat pendant and tends to have an edge separating a relatively smooth outer face from a very coarsely and boldly papillate inner surface; in *sucklii* the upper lip is more strongly and more uniformly swollen and rounded, shows almost no differentiation of an outer and an inner face, and is remarkably uniform in papillation. The cartilaginous ridges or biting edges of the jaws in *griseus* (again approaching *Pantosteus*) are much stronger, and are more transverse and wider than in *sucklii*—in which these ridges are rudimentary. The tangible differences in mouth structure separate *griseus* widely from *sucklii*, leaving a gap into which the hybrids fit.

The 5 specimens also give evidence of hybridity in coloration. The young of *sucklii* have 3 large and rather intense dark blotches along the side of the body, one close to the head, another below the dorsal fin, and the third near the base of the caudal fin (Pl. IV, Fig. 1). These same marks are evident in *griseus* (as in many other suckers), but in that species are smaller and less conspicuous and show a greater tendency to grade into the general dark marbling on the upper sides (which is more prominent than in *sucklii*). In *sucklii* the pattern is less sharply bicolored, for the dark upper parts tend to contrast less markedly and less abruptly with the white lower sides. The individual scale pockets of *sucklii* are usually the more sharply set off, by light centers and by dark-lined borders (Pl. III). All these characters vary with size, but the relative differences persist through most of the life cycle. When the hybrids are compared with specimens of the parent species of like size, the intermediate pigmentation is apparent.

The life colors were also found to be transitional. According to our field notes the half-grown specimen from Laramie River had the dorsal shade of intervening darkness. Intermediacy was also seen in the slight golden tinge of the lower surfaces, and in the slightly yellowish lips. The lower fins had an intermediate amount of red: they were brighter than the pale dirty amber of *sucklii,* duller than the bright orange amber of *griseus.*

Considering all characters, we are convinced by the evidence that *Catostomus commersonnii sucklii* and *Catostomus catostomus griseus* occasionally mate in nature to produce hybrids.

2. *Catostomus macrocheilus* × *Catostomus syncheilus syncheilus*

(Pl. II, Fig. 1*b*; Pl. VI, Fig. 1*b*)

U.M.M.Z. No. 98651: 3 large young 36.5 to 46.5 mm. in standard length, collected by Leonard P. Schultz and Marvin Bowers in sloughs of Wenatchee River at its confluence with Columbia River, near Wenatchee, Chelan County, Washington; August 23, 1932.

U.M.M.Z. No. 98736: 1 young 29 mm. long, seined by Schultz and Bowers in Grande Ronde River in Asotin County, Washington, between Antone, Washington, and Paradise, Oregon; August 29, 1932.

U.M.M.Z. No. 98757: 3 young to half-grown specimens, 35.5, 95, and 113 mm. long, taken by the same collectors in Grande Ronde River 1 mile above Elgin, Union County, Oregon; August 30, 1932.

U.M.M.Z. No. 98761: 3 young 33 to 41 mm. long, obtained on the same expedition in Grande Ronde River at Perry, Union County, Oregon; August 30, 1932.

U.M.M.Z. No. 98776: 37 young 28.5 to 44 mm. in length, seined by Schultz and Bowers in a slough and on the riffles of Umatilla River at Rieth, below Pendleton, Oregon; August 30–31, 1932.

U.M.M.Z. No. 98777: An adult 190 mm. long, taken by the same party in Umatilla River at Echo, Oregon; August 31, 1932.

U.M.M.Z. No. 98793: 7 large young to half-grown, 47.5 to 108 mm. long, secured by Schultz and Bowers in Umatilla River at its confluence with the Columbia; August 31 and September 1, 1932.

U.M.M.Z. No. 130471: 9 yearling and half-grown fish, 60 to 150 mm. in standard length, obtained by Carl L. Hubbs and family in Sucker (= Succor) Creek, a tributary of Snake River, above Rockville, Malheur County, Oregon, about 4 miles from the Idaho line (Town 24 S., Range 46 E.); July 24, 1934.

U.M.M.Z. No. 130482: 1 half-grown of 142 mm., seined on the same trip in Jordan Creek, a tributary of Owyhee River, at Ruby Ranch about midway between Jordan Valley and Rome, Malheur County, Oregon (T. 30 S., R. 44 E.); July 24, 1934.

U.M.M.Z. No. 131787: 1 yearling 35 mm. long, seined by Leonard P. Schultz and Leo Erkkila in Crab Creek 1 mile above Beverly Slough (formed by flood water from the Columbia River); June 19, 1932.

U.M.M.Z. No. 131788: 2 yearlings, 34 and 36 mm. in length, taken by the same collectors in Crab Creek at Smyrna; June 19, 1932.

U.M.M.Z. No. 138616: 1 young 36 mm. long, collected by Stanley G. Jewett, Jr., and Edwin Niska about 1 mile above Umatilla River dam; September 17, 1940. The counts and measurements of this specimen are intermediate, but it was discovered too late for inclusion in the tabulations.

TABLE IV

Relative Numbers of Hybrids, *Catostomus macrocheilus* × *Catostomus syncheilus syncheilus*, and of the Parental Kinds

Specimens in University of Michigan Museum of Zoology	*Catostomus macrocheilus*	Hybrids	*Catostomus syncheilus syncheilus*
In collections containing hybrid			
U.M.M.Z. No. 98651	73	3	0
U.M.M.Z. No. 98736	20	1	0
U.M.M.Z. No. 98757	8	3	39
U.M.M.Z. No. 98761	0	3	3
U.M.M.Z. No. 98776	120	37	88
U.M.M.Z. No. 98777	16	1	3
U.M.M.Z. No. 98793	116	7	3
U.M.M.Z. No. 130471	6	9	125
U.M.M.Z. No. 130482	3	1	3
U.M.M.Z. No. 131787	13	1	0
U.M.M.Z. No. 131788	0	2	6
U.M.M.Z. No. 138616	9	1	3
Total	384	69	273
From the Columbia River system	969	69	1341*

* In this total we include all available material of *syncheilus* other than the Palouse River population of *C. syncheilus palouseanus*, though other subspecies are seemingly included.

The failure to get both parental species at 5 of the 12 localities where hybrids were secured is not very surprising. Hybrids Nos. 98651 and 131787 were collected in sloughs where *macrocheilus* but not *syncheilus* would be at home. *Catostomus syncheilus* shows a greater preference for smaller and more rapid waters. *C. macrocheilus* is widespread ecologically, though the adults and many young tend to drop back into quiet pools and bayous. In the spring spawning season both species no doubt seek out gravelly stretches in creeks, as this is the usual spawning locale for suckers in general (Reighard, 1920). Schultz (1941: 28) definitely stated that *C. macrocheilus* "spawns in the spring, usually in April and May, when large numbers migrate up the stream and deposit their eggs on the gravel riffles in swiftly flowing water." The fact that either the one or the other parent species only was taken with hybrids Nos. 98736, 98761, and 131788 may be attributed to chance and to the small size of the collections, for both parental forms occur in similar habitats in the immediate vicinity.

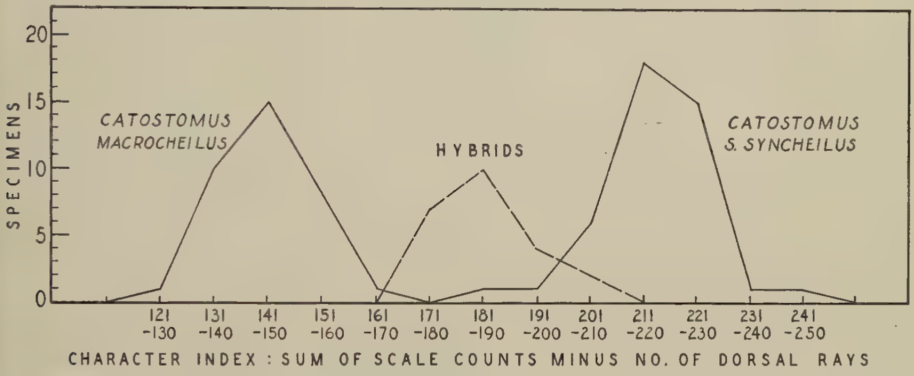

FIG. 1. Comparison of hybrids, *Catostomus macrocheilus* × *Catostomus s. syncheilus*, with the parental species in a character index involving scale and ray counts (Table V).

Until recently the status of the forms of *Catostomus* in the Columbia River system was very much confused, but it now appears clear that 3 species are represented there (Schultz, 1941: 27–30). Of these, *Catostomus catostomus* (as subspecies *pocatello* and either *catostomus* or *griseus*) is largely confined in this drainage basin to the Rocky Mountain region. More widespread are (1) the well-known, coarse-scaled *C. macrocheilus,* and (2) a form long confused with *C. catostomus,* though now known to be quite distinct. This second species is *Catostomus syncheilus* Hubbs and Schultz (1932). The differential features of *C. macrocheilus* and *C. syncheilus* should be kept clearly in mind, for these are obviously the species which hybridize with considerable frequency.

On the average, the hybrid indices for each individual of the *C. macrocheilus* × *C. syncheilus syncheilus* combination show the expected interspecific intermediacy: the average value for the 68 hybrid specimens is 53 (Table XXXIX). Approximately half (33) of the specimens have the index between 45 and 75. The considerable spread is probably to be attributed less to the true variability of the hybrids than to the small size of most of the examples at hand. Probably few if any of the specimens are misjudged to be hybrids, but on account of the small size the differences in proportions are minimized and the counts and measurements are subject to considerable error.

The interposition of the hybrids is again illustrated by the counts. In the number of dorsal rays and in the counts of scales in the several series, the hybrids bridge over the gap between the parental species (Tables XXXII to XXXVII; Fig. 7). There is considerable overlap in each of the comparisons (without, however, a marked increase in variability). The transgression of the hybrid characters on those of the 2 species is almost wholly eliminated when the counts are combined into a character index, similar (but not quite identical) to that used by Schultz and Thompson (1936: 74–75), in the distinction of *Catostomus syncheilus palouseanus*. The index as here used is obtained by adding all the scale counts (which tend to be higher in *syncheilus*) and then subtracting from this sum the dorsal ray count

TABLE V

CHARACTER INDICES (SUM OF ALL SCALE COUNTS MINUS NUMBER OF DORSAL RAYS) IN HYBRIDS BETWEEN *Catostomus macrocheilus* AND *Catostomus syncheilus*, AND IN THE PARENTAL SPECIES

Character Index	Columbia River System (Excluding Palouse Division)			Palouse River System		
	Catostomus macrocheilus	Hybrids	*Catostomus syncheilus syncheilus*	*Catostomus macrocheilus*	Hybrids	*Catostomus syncheilus palouseanus*
121–130	1	....	....	1	....	....
131–140	10	....	....	1	....	....
141–150	15	....	....	15	....	....
151–160	8	....	....	7	....	....
161–170	1	....	....	4	....	....
171–180	....	7	....	1	....	....
181–190	....	10	1	....	1	....
191–200	....	4	1	....	....	1
201–210	....	2	6	....	1	....
211–220	....	....	18	....	....	7
221–230	....	....	15	....	....	7
231–240	....	....	1	....	....	12
241–250	....	....	1	....	....	2
251–260	....	....	....	....	....	1
Specimens*	35	23	43	29	2	30
Average†	145	186	218	150	197	229
Standard error†	1.4	1.8	1.6	1.8	....	2.0
V	5.7	4.6	4.8	6.5	....	4.8

* The number of specimens is lower than in other tables because all of the scale counts could not be taken accurately in some of the smallest fish.

† Computed from the original, unclassed data.

(which tends to be higher in *macrocheilus*). The results, for the 2 *macrocheilus* × *syncheilus* comparisons, are given in Table V and Figure 1. The frequency distribution for the hybrids is unimodal and essentially normal, and again there is no increase in variability.

Similar intermediacy is exhibited by the proportionate measurements (Table VI), though less strikingly than for the counts because of the greater variation and greater overlap in the dimensions. Definite intermediacy for all or nearly all of the comparisons (broken down by locality and by size group) is indicated for the length and depth of the head, the width of the mouth and of the cartilaginous ridge, the length of the union of the lower lip (except in young fish), the distance from the dorsal fin to the occiput, and, particularly well, for the basal length and the depressed length of the dorsal fin. A slight tendency toward intermediacy is shown for the depth of the caudal peduncle. The 4 other measurements (body depth, head width, snout length, and mouth length) are not critical.

As pointed out earlier, hybridity is not demonstrated for a group of specimens by an intermediate average measurement for any part of the body, since a mixture of specimens of the 2 supposed parental species would also yield an intervening average. Hybridization without any clear-cut Mendelian segregation will be indicated when the specimens in question have a unimodal frequency distribution about an intermediate mean. Unequivocal testimony of this sort is difficult to obtain for measured characters, because almost all the proportions vary so much with age and locality that any reasonable units of comparison ordinarily contain too few specimens to show the true nature of the frequency distributions. Inspection of the relatively abundant data for the *C. macrocheilus* × *C. s. syncheilus* cross, however, indicates that the hybrids tend to be intermediate in the proportionate measurements, with a clustering of the values about the intermediate means.

Two of the more significant measurements have been combined into a simple character index to test the frequency distribution of the resulting proportion about the intermediate mean. This index is:

$$\frac{\text{Distance from origin of dorsal fin to occiput}}{\text{Length of depressed dorsal fin}}$$

It is applied to young specimens 25 to 63 mm. in standard length for 2 reasons:

(1) The hybrid nature of small specimens is in special need of confirmation—the hybridity of the larger individuals is more palpably evident.

(2) The index is consistent only in the young. Some of the larger specimens of each group (*macrocheilus*, hybrids, *syncheilus*) have the index about as in the young; others have a smaller fin, giving a higher index. Either sexual dimorphism or a differential attainment of maturity at comparable sizes introduces a complexity. Between the lengths of 25 and 63 mm. analysis shows no significant progressive change in the index. Therefore, the data for this size range only are considered. It is clear from the data (Table VII

TABLE VI

Proportionate Measurements of Hybrids between *Catostomus macrocheilus* and *Catostomus syncheilus syncheilus*, and of the Parental Forms

The measurements are expressed as thousandths of the standard length. There are given for each item the range of the measurements; the average, in parentheses; and, as a subscript, the number of specimens.

	Umatilla River, Oregon			Wenatchee River and Lower Crab Creek, Washington	Grande Ronde River, Oregon and Washington		Jordan Creek, Oregon	Sucker Creek, Oregon
Size group, mm.								
C. macrocheilus	28–52 $(36)_{30}$	88–110 $(97)_{8}$	128–256 $(198)_{7}$	35–46 $(42)_{10}$	26–38 $(32)_{9}$	102–168 $(130)_{3}$	77–122 $(106)_{3}$	72–149 $(102)_{6}$
Hybrids	28–48 $(35)_{38}$	80–107 $(94)_{6}$	.... $(190)_{1}$	34–46 $(39)_{6}$	29–40 $(35)_{5}$	95–113 $(104)_{2}$	.... $(146)_{1}$	60–150 $(93)_{9}$
C. s. syncheilus	29–44 $(36)_{28}$	72–91 $(83)_{3}$	....	33–36 $(34)_{8}$	25–40 $(32)_{3}$	74–96 $(86)_{10}$	137–173 $(161)_{3}$	59–166 $(102)_{25}$
Body depth								
C. macrocheilus	171–221 (199)	195–225 (216)	184–231 (212)	191–221 (204)	166–212 (193)	196–226 (209)	208–215 (211)	206–227 (215)
Hybrids	174–216 (194)	215–236 (226)	.... (218)	189–217 (201)	180–195 (186)	201–212 (207)	.... (204)	191–233 (211)
C. s. syncheilus	167–210 (194)	205–225 (217)	....	182–223 (200)*	189–198 (194)	191–218 (205)	195–204 (199)	168–229 (198)
Caudal peduncle, depth								
C. macrocheilus	66–76 (71)	65–75 (71)	71–84 (77)	68–77 (75)	67–75 (71)	75–81 (78)	71–79 (75)	71–83 (77)
Hybrids	69–81 (75)	73–78 (75)	.... (81)	63–84 (77)	67–80 (75)	79–79 (79)	.... (77)	76–81 (78)
C. s. syncheilus	67–79 (74)	78–83 (81)	....	69–86 (78)	70–77 (73)	77–83 (81)	78–79 (78)	67–82 (77)
Head length								
C. macrocheilus	259–303 (282)	248–272 (259)	233–261 (249)	261–286 (276)	273–301 (290)	233–240 (237)	246–269 (259)	254–267 (259)
Hybrids	249–294 (272)	236–245 (241)	.... (242)	257–297 (271)	255–283 (270)	232–250 (241)	.... (238)	233–268 (255)
C. s. syncheilus	250–281 (265)	238–242 (239)	....	264–273 (269)	259–268 (265)	240–255 (247)	212–227 (220)	226–259 (244)

* Computations based on measurements of 7 specimens.

Head depth								
C. macrocheilus	150–186 (176)	161–172 (167)	156–169 (162)	169–190 (179)	167–185 (177)	152–153 (153)	164–171 (168)	159–172 (164)
Hybrids	158–184 (171)	151–161 (155)	(152)	165–186 (174)	166–183 (173)	151–158 (155)	(146)	145–172 (158)
C. s. syncheilus	159–175 (166)	146–153 (149)		161–174 (169)	165–178 (170)	151–159 (154)	132–137 (135)	135–162 (150)
Head width								
C. macrocheilus	140–158 (149)	151–163 (156)	151–165 (155)	139–157 (148)	148–168 (156)	145–151 (148)	152–158 (155)	147–162 (152)
Hybrids	137–164 (152)	151–155 (153)	(145)	148–161 (151)	145–160 (152)	149–154 (151)	(146)	147–160 (151)
C. s. syncheilus	140–160 (151)	150–157 (154)		126–146 (140)	142–158 (152)	143–155 (150)	139–144 (142)	141–159 (149)
Snout length								
C. macrocheilus	85–120 (99)	111–121 (115)	96–122 (113)	87–100 (96)	99–112 (103)	109–121 (114)	105–116 (111)	112–132 (119)
Hybrids	75–115 (95)	105–115 (110)	(118)	87–112 (100)	86–118 (97)	104–109 (107)	(113)	100–113 (108)
C. s. syncheilus	82–111 (97)	91–119 (104)		86–101 (93)	86–96 (93)	109–124 (114)	96–106 (102)	96–117 (107)
Mouth width								
C. macrocheilus	57–88 (71)	62–75 (70)	68–80 (76)	61–73 (68)	64–73 (68)	68–73 (70)	68–70 (69)	75–91 (80)
Hybrids	62–85 (74)	71–83 (78)	(85)	69–81 (74)	68–82 (75)	71–74 (73)	(69)	79–92 (86)
C. s. syncheilus	66–85 (76)	79–91 (86)		61–72 (66)*	68–82 (76)	82–103 (94)	76–83 (80)	81–97 (90)
Cartilaginous ridge, width								
C. macrocheilus	30–43 (39)	32–39 (35)	32–41 (35)	35–44 (38)	37–50 (45)	33–44 (39)	35–38 (37)	41–48 (44)
Hybrids	34–50 (44)	41–56 (46)	(48)	42–51 (44)	41–48 (44)	42–42 (42)	(47)	40–53 (48)
C. s. syncheilus	41–50 (46)	49–52 (50)		42–49 (45)	40–49 (43)	46–57 (53)	51–53 (52)	46–60 (55)

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* Computations based on measurements of 7 specimens.

TABLE VI—(*Cont.*)

	Umatilla River, Oregon			Wenatchee River and Lower Crab Creek, Washington	Grande Ronde River, Oregon and Washington		Jordan Creek, Oregon	Sucker Creek, Oregon
Mouth length								
C. macrocheilus	43–62 (54)	48–61 (53)	54–69 (63)	46–54 (50)	46–53 (50)	52–62 (58)	49–60 (55)	61–76 (68)
Hybrids	48–62 (55)	49–60 (55)	.... (66)	48–57 (52)	46–57 (55)	52–56 (54)	.... (59)	61–76 (70)
C. s. syncheilus	42–70 (57)	54–62 (59)	....	47–52 (49)	54–56 (55)	61–66 (63)	54–57 (55)	59–73 (66)
Lower lip, union								
C. macrocheilus	9–16 (12)	9–13 (11)	9–16 (12)	8–12 (10)	7–15 (12)	15–16 (16)	8–11 (9)	15–20 (18)
Hybrids	10–16 (13)	12–26 (17)	.... (19)	8–13 (11)	10–13 (12)	14–18 (16)	.... (17)	17–26 (21)
C. s. syncheilus	10–22 (14)	23–24 (23)	....	10–14 (12)	9–16 (14)	17–30 (23)	22–24 (23)	18–30 (23)
Dorsal fin to occiput, distance								
C. macrocheilus	237–282 (256)	286–316 (304)	307–323 (315)	225–256 (246)	248–267 (255)	289–294 (292)	265–283 (273)	236–299 (261)
Hybrids	246–296 (269)	300–324 (315)	.... (321)	240–270 (255)	243–272 (260)	275–280 (277)	.... (289)	252–290 (275)
C, s. syncheilus	251–316 (276)	318–335 (327)	....	253–275 (266)	264–276 (270)	263–321 (293)	292–309 (302)	264–321 (290)
Dorsal fin base								
C. macrocheilus	195–253 (224)	198–235 (214)	198–215 (207)	219–255 (243)	222–243 (232)	193–211 (200)	227–244 (234)	203–228 (220)
Hybrids	187–228 (205)	184–221 (201)	.... (186)	195–242 (218)	203–241 (213)	193–205 (199)	.... (203)	198–227 (210)
C. s. syncheilus	178–212 (191)	176–186 (182)	....	160–203 (189)	187–219 (201)	172–204 (185)	173–184 (178)	166–212 (193)
Depressed dorsal fin, length								
C. macrocheilus	268–326 (305)	287–305 (293)	273–321 (297)	301–342 (330)	298–328 (314)	284–295 (290)	330–343 (335)	290–328 (318)
Hybrids	274–315 (292)	268–304 (281)	.... (271)	291–330 (310)	280–330 (296)	284–298 (291)	.... (294)	296–322 (311)
C. s. syncheilus	258–293 (278)	261–269 (266)	....	255–302 (279)	278–291 (283)	255–285 (275)	257–280 (265)	246–315 (289)

and Fig. 2) that the indices for hybrids have an intervening mean, have the same order of variability, and form an approximately normal unimodal frequency distribution. Their identification as hybrids is confirmed and no simple Mendelian segregation is suggested. The data for other measurements, though analyzed in less detail, seem to show the same sort of statistics; as do the dorsal-ray and scale counts (compare Table VII with Tables XXXII to XXXVII).

A further confirmation of the hybrid interpretation is provided by the pelvic-ray count—a character discovered after the paper was written. Again we note intermediacy with no increase in variability (Table VIII).

The intermediacy of the hybrids is well shown also in characters that are not so readily analyzed as are the counts and the ordinary proportions.

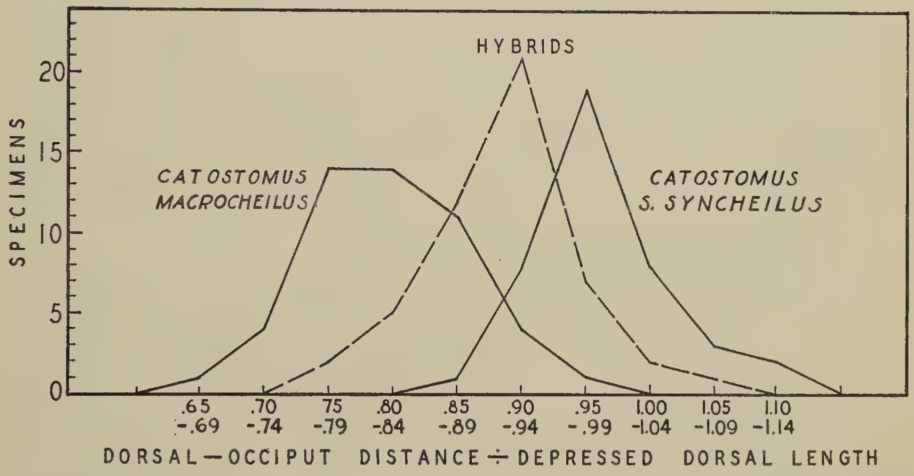

FIG. 2. Comparison of hybrids, *Catostomus macrocheilus* × *Catostomus s. syncheilus*, with the parental species in the ratio of the dorsal–occiput distance to the depressed dorsal length (Table VII).

Thus the snout tends to be less depressed and less prominently projecting beyond the mouth than in *syncheilus*, but more so than in *macrocheilus;* the hybrids tend to be intermediate (Pl. VI, Figs. 1*a*–*c*).

As in most sucker hybrids, the mouth structures also exhibit intermediate characters (as shown in Pl. II, Figs. 1*a*–*c;* compare also Figs. 2*a*–*c* on Pl. VI). The intermediacy is shown in every one of the following features, by which the lips of *syncheilus* differ from those of *macrocheilus:*

1. The lips are broader and flatter.
2. The upper lip is less gibbous and more pendant, especially around the front of the mouth. It has a definite edge (scarcely represented in *macrocheilus*), which separates an outer nearly smooth face from an inner strongly papillate one. In *macrocheilus* there is only an incipient differentiation of this sort.

TABLE VII

Frequency Distribution of the Ratio of the Distance from the Origin of the Dorsal to the Occiput, to the Length of the Depressed Dorsal Fin, in Hybrids between *Catostomus macrocheilus* and *Catostomus syncheilus syncheilus*, and in the Parental Kinds

Measured in young specimens, 25 to 63 mm. in standard length.

	Dorsal to Occiput ÷ Depressed Dorsal Length									
	0.65–0.69	0.70–0.74	0.75–0.79	0.80–0.84	0.85–0.89	0.90–0.94	0.95–0.99	1.00–1.04	1.05–1.09	1.10–1.14
C. macrocheilus	1	4	14	14	11	4	1	......	......	......
Hybrids	......	......	2	5	12	21	7	2	1	......
C. s. syncheilus	......	......	......	......	1	8	19	8	3	2

	Statistical Computations					
	No.	Range	Ave.*	S.E.*	V	Hybrid Index with Standard Error
C. macrocheilus	49	0.66–0.97	0.82	.009	7.7	....
Hybrids	50	0.79–1.02	0.91	.008	6.4	56 ± 6
C. s. syncheilus	41	0.88–1.12	0.98	.008	5.4	....

* The averages and standard errors were computed from the original unclassed data.

TABLE VIII

Number of Pelvic Rays in Hybrids between *Catostomus macrocheilus* and *Catostomus syncheilus*, and in the Parental Species*

Region and Kind	Number of Rays in Both Pelvic Fins						
	18	19	20	21	22	23	24
Exclusive of Palouse River system							
C. macrocheilus	......	......	7	12	64	6	4
Hybrids	......	1	21	12	28	2	......
C. s. syncheilus	2	7	42	26	31	......	1
Palouse River system							
C. macrocheilus	......	1	1	4	31	4	7
Hybrids	......	......	1	2	......	......	......
C. s. palouseanus	1	2	27	11	14	......	......

	Statistical Computations				
	No.	Ave.	S.E.	V	Hybrid Index ± S.E.$_I$
Exclusive of Palouse River system					
C. macrocheilus	93	21.87	.11	4.9	....
Hybrids	64	21.14	.12	4.6	65 ± 14
C. s. syncheilus	109	20.74	.10	5.1	....
Palouse River system					
C. macrocheilus	48	22.19	.14	4.5	....
Hybrids	3	20.67?	.33?	2.3?	98?
C. s. palouseanus	55	20.64	.13	4.65	....

* These data were obtained too late to be used in the subsequent computations of hybrid intermediacy.

3. At the outer angle of the mouth where the 2 lips are joined, a slight to moderately deep indentation is often developed, at the end of a crease between the upper and lower lips (this feature is hardly shown by the specimen drawn for Fig. 1*c*, Pl. II). In *macrocheilus* this groove is less well developed and does not reach the edge of the mouth, and there is no emargination at the angle.

4. The median incision between the lobes of the lower lips is less developed. The 2 halves of the lip are connected by a flatter and broader isthmus, across which there pass 2 to 5 instead of 1 to 2 rows of papillae.

5. The cartilaginous ridges of the mouth are much more strongly developed, wider, and less curved (more nearly transverse). This development approaches that of *Pantosteus*, but is much less extreme.

Similar intermediacy is exhibited in the coloration. *Catostomus syncheilus* is darker than *macrocheilus*. The fine dark mottlings of the upper parts tend to be more prominent. The dark color usually extends farther down across the lower sides and tends to grade into the light lower surface, rather than to give way somewhat abruptly to a light ventrolateral area on the head and body. In all these respects the hybrids tend to be transitional between the parental species.

A few specimens which we have interpreted as hybrids, particularly in the collections of young, may well belong to either the one or the other of the parental species, and a few diagnosed as the pure species may be hybrids. Most of the determinations, however, seem correct.

We conclude that *Catostomus macrocheilus* and *Catostomus syncheilus syncheilus* hybridize rather commonly. A rather uniform intermediacy pervades all the known distinguishing features: number of dorsal and of pelvic fin rays; number and size of scales on all parts of the body; differences in proportions and form; distinctive characters in the structure of the mouth; differences in coloration. The intermediacy in any one of these systems would suggest hybridization; the intermediacy in all respects virtually clinches the conclusion. By any other theory it would be difficult to explain why the specimens intermediate in counts (Fig. 1) should also show intervening proportions (Fig. 2). The multiple-factor type of inheritance is indicated for all the systematic differentiae.

3. *Catostomus macrocheilus* × *Catostomus syncheilus palouseanus*

(Pl. VI, Fig. 2*b*)

U.M.M.Z. No. 98709: 1 yearling 65 mm. and 1 adult 204 mm. in standard length, seined by Leonard P. Schultz and Marvin Bowers in South Fork of Palouse River, 0.5 mile above Pullman, Washington; August 27, 1932.

U.M.M.Z. No. 98725: 1 young fish 29 mm. long taken by the same collectors on the following day in the Palouse River at Palouse, Washington.

In considering the relative numbers of hybrids and parental species in this combination, and in that of *C. macrocheilus* × *C. s. syncheilus*, it should be recalled that parts of the collections made by Leonard P. Schultz and assistants were retained at the University of Washington.

The interpretation of 3 individuals as hybrids between *C. macrocheilus* and *C. syncheilus palouseanus* is on the same basis as the identification of 69 specimens as hybrids between *macrocheilus* and the typical subspecies of *syncheilus*.

We have found in the Cyprinidae, Poeciliidae, and other families that the hybrids in the same interspecific combination differ, when different subspecies of one (or both) parental species are involved. The difference is in the expected direction, on the assumption that the heterozygotic forms tend to be more or less precisely intermediate between the particular parental types. When a subspecies of a parental species has some peculiarity in counts, measurements, or color, this feature is generally to be seen, in diluted form, in the hybrid. This circumstance is part of the large body of data which convinces us of the validity of the assumption that species of fish hybridize in nature.

TABLE IX

Relative Numbers of Hybrids, *Catostomus macrocheilus* × *Catostomus syncheilus palouseanus*, and of the Parental Kinds

Specimens in University of Michigan Museum of Zoology	*Catostomus macrocheilus*	Hybrids	*Catostomus syncheilus palouseanus*
In collections containing hybrid			
U.M.M.Z. No. 98709	72	2	57
U.M.M.Z. No. 98725	21	1	3
Total	93	3	60
From the Palouse River system	152	3	358

These conclusions are confirmed by the limited data on the hybrids involving *C. s. palouseanus;* unfortunately only 3 specimens have been identified and 1 of these is too small for the determination of some characters. As compared with the hybrids between *C. macrocheilus* and typical *C. s. syncheilus,* those between *C. macrocheilus* and *C. s. palouseanus* have a lower average number of dorsal rays, and a higher average number of scales; *palouseanus* differs from typical *syncheilus* in the same way (as first shown by Schultz and Thompson, 1936), and the Palouse River race of *C. macrocheilus* seems to show a local variation parallel to that exhibited by *C. s. palouseanus.* These relations are perhaps brought out best by the character index involving dorsal ray and scale counts (Table V).

That the 3 hybrids of this combination are nicely intermediate is indicated by their average hybrid indices (Table XXXIX) of 50, 57, and 60, on the percentage scale in which 50 denotes exact intermediacy in all the characters studied.

TABLE X

PROPORTIONATE MEASUREMENTS OF HYBRIDS BETWEEN *Catostomus macrocheilus* AND *Catostomus syncheilus palouseanus*, AND OF THE PARENTAL SPECIES

The measurements are expressed as thousandths of the standard length. There are given for each item the range of the measurements; the average, in parentheses; and, as a subscript, the number of specimens.

	Palouse River System, Washington		
Size group, mm.			
C. macrocheilus	30–42 (36)$_{25}$	44–105 (80)$_{18}$	120–255 (195)$_{7}$
Hybrids	…… (29)$_{1}$	…… (65)$_{1}$	…… (204)$_{1}$
C. syncheilus palouseanus	33–40 (36)$_{13}$	44–85 (65)$_{14}$	122–178 (140)$_{15}$
Body depth			
C. macrocheilus	177–215 (197)	189–236 (213)	203–221 (213)
Hybrids	…… (207)	…… (198)	…… (198)
C. syncheilus palouseanus	186–243 (221)	183–247 (219)	193–217 (205)*
Caudal peduncle, depth			
C. macrocheilus	65–79 (74)	68–81 (73)	72–83 (79)
Hybrids	…… (90)	…… (79)	…… (79)
C. syncheilus palouseanus	71–86 (81)	77–84 (81)	75–86 (81)
Head length			
C. macrocheilus	271–306 (287)	254–286 (269)	244–265 (253)
Hybrids	…… (274)	…… (274)	…… (243)
C. syncheilus palouseanus	252–275 (266)	232–270 (251)	218–254 (231)
Head depth			
C. macrocheilus	173–188 (181)	162–181 (173)	163–172 (167)
Hybrids	…… (176)	…… (166)	…… (150)
C. syncheilus palouseanus	161–182 (169)	153–176 (160)	134–155 (143)
Head width			
C. macrocheilus	148–164 (156)	145–164 (156)	154–169 (162)
Hybrids	…… (159)	…… (173)	…… (163)
C. syncheilus palouseanus	142–166 (157)	136–164 (151)	145–166 (152)
Snout length			
C. macrocheilus	91–122 (109)	95–131 (120)	112–141 (131)
Hybrids	…… (99)	…… (114)	…… (125)
C. syncheilus palouseanus	94–115 (104)	93–118 (108)	91–123 (113)

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* Computations based on measurements of 14 specimens.

TABLE X—(*Cont.*)

PROPORTIONATE MEASUREMENTS OF HYBRIDS BETWEEN *Catostomus macrocheilus* AND *Catostomus syncheilus palouseanus*, AND OF THE PARENTAL SPECIES

	Palouse River System, Washington		
Mouth width			
C. macrocheilus	68–85 (75)	64–83 (76)	67–102 (85)
Hybrids	…… (77)	…… (91)	…… (78)
C. syncheilus palouseanus	73–92 (80)	73–104 (86)	81–107 (94)
Cartilaginous ridge, width			
C. macrocheilus	38–55 (42)	36–44 (40)	35–46 (41)
Hybrids	…… (50)	…… (48)	…… (43)
C. syncheilus palouseanus	31–46 (43)	40–57 (49)	45–57 (51)
Mouth length			
C. macrocheilus	47–65 (55)	51–64 (58)	57–77 (70)
Hybrids	…… (55)	…… (65)	…… (60)
C. syncheilus palouseanus	52–60 (56)	47–75 (61)	64–77 (69)
Lower lip, union			
C. macrocheilus	8–17 (12)*	9–16 (12)	13–20 (15)
Hybrids	…… (17)	…… (17)	…… (17)
C. syncheilus palouseanus	11–19 (17)	15–25 (19)†	16–30 (23)
Dorsal fin to occiput, distance			
C. macrocheilus	235–264 (250)	233–276 (256)	250–300 (282)
Hybrids	…… (227)	…… (266)	…… (287)
C. syncheilus palouseanus	244–272 (261)	263–292 (279)	251–320 (284)
Dorsal fin base			
C. macrocheilus	184–237 (215)	192–242 (215)	179–212 (190)
Hybrids	…… (197)	…… (186)	…… (176)
C. syncheilus palouseanus	182–208 (191)	169–222 (187)	156–199 (178)
Depressed dorsal fin, length			
C. macrocheilus	282–327 (302)	281–332 (301)	252–298 (272)
Hybrids	…… (286)	…… (281)	…… (248)
C. syncheilus palouseanus	259–294 (275)	255–293 (274)†	221–304 (264)

* Computations based on measurements of 24 specimens.
† Computations based on measurements of 13 specimens.

The intermediate features of *C. macrocheilus* × *C. syncheilus palouseanus* hybrids involve proportionate measurements as well as the counts. As shown by Table X, either 2 or all of the 3 hybrids are intermediate between specimens of the parental types of comparable sizes in: the length and depth of the head, the length of snout, the width of the cartilaginous ridge in the mouth, the length of the union of the lower lip (Pl. VI, Figs. 2*a–c*), and the length of the depressed dorsal fin. Inconsistent results were obtained for the depth of the body and of the caudal peduncle, the width of the head and of the mouth, the distance between the origin of the dorsal fin and the occiput, and the length of the base of the dorsal fin.

The interpretation of hybrid origin and of the seemingly Galtonian inheritance of the systematic characters is confirmed by the form of the snout, the structure of the mouth, and the coloration (Pl. VI, Figs. 2*a–c*). The remarks on these features given for the *C. macrocheilus* × *C. s. syncheilus* cross apply *in toto*.

HYBRIDS BETWEEN THE GENERA *CATOSTOMUS* AND *PANTOSTEUS*

With even greater confidence we can claim that species of *Catostomus* and of *Pantosteus* hybridize in nature. In these combinations, intergeneric according to the current nomenclature of the family, the gap between the parental species is so wide and involves so many characters that specimens which are intermediate in all important respects can hardly be interpreted in any other light. Many of the differences which distinguish *C. catostomus* from *C. commersonnii*, and *C. syncheilus* from *C. macrocheilus* appear exaggerated when we contrast species of *Pantosteus* with those of *Catostomus*. The snout of *Pantosteus* is much more depressed and bulbous and projects beyond the mouth (Pls. IV to VII). The upper lip (Pls. I and IV) in *Pantosteus* is even more modified than in *Catostomus catostomus* and *C. syncheilus*, in the better developed ridge and in the overhanging form, with the smoother outer part tending to overlap the strongly papillate inner surface. The lips are flatter. The crease separating the upper and lower lips on each side is deeper and extends to the angle of the rather deep emargination at the outer corner of the mouth. On the midline, in contrast, the lower lips are much more broadly conjoined. The cartilaginous biting or scraping edge of the lower jaw is much better developed. This ridge is stronger, broader, and much more nearly transverse. The gape in consequence is straightish, rather than strongly arched. The outer ends of the ridge are more definitely shouldered. In some species of *Catostomus*, notably *C. commersonnii*, the mouth is moderately oblique, whereas in *Pantosteus* it is always strictly inferior and horizontal (Pls. IV–V). In *Pantosteus* the general color is darker, and the peritoneum is more jet-black than in the species of *Catostomus* with which it has been found to hybridize (the differences in peritoneal pigment are especially notable in the young, in which the black color often shows through the thin belly wall). The intestine averages longer, and the air bladder more reduced in size.

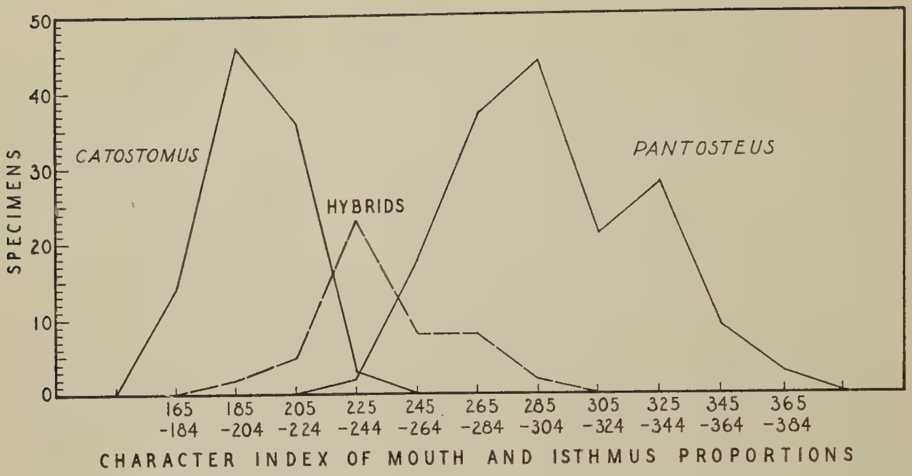

FIG. 3. Comparison of all *Catostomus* × *Pantosteus* hybrids with the parental species in the character index of mouth and isthmus proportions.

The concordant data of 1942 for the *Catostomus*, species × *Pantosteus santa-anae* cross are included in Tables XI and XII, but not in this graph.

In all these respects the hybrids correspond with neither parent species, but are intermedial. The hybrids of the different *Catostomus* × *Pantosteus* combinations are not exactly alike, nor would this be expected, for the parental species of the several combinations are diverse. Thus in mouth structure the special features of the parent species of the different combinations are reflected by the hybrids (Pls. I and IV).

That the mouths of the *Catostomus* × *Pantosteus* hybrids are intermediate in measurements as well as in structural details is brought out by the data in Tables XI and XII and in Figures 3 and 4. In order to restrict the overlap of the hybrid values on those of the parental types and to simplify

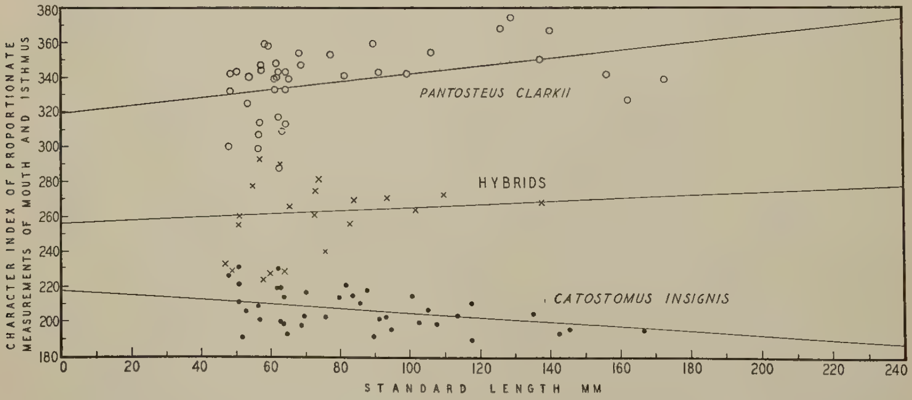

FIG. 4. Variation with standard length of the character index involving mouth and isthmus proportions in hybrids, *Catostomus insignis* × *Pantosteus clarkii*, and in the parental species.

TABLE XI

FREQUENCY DISTRIBUTION, IN *Catostomus* × *Pantosteus* HYBRIDS, OF THE CHARACTER INDEX OF MOUTH AND ISTHMUS MEASUREMENTS

The index represents the sum of the proportionate measurements, expressed in thousandths of the standard length, of (1) the width of the mouth, (2) the width of the cartilaginous ridge of the mouth, (3) the length of the union of the lower lip, and (4) the width of the isthmus.

	Character Index Frequencies											Statistical Computations					
	165–184	185–204	205–224	225–244	245–264	265–284	285–304	305–324	325–344	345–364	365–384	No.	Range	Ave.	S.E.	V	Hybrid Index
C. c. sucklii	4	5	1	......	......	......	......	......	......	......	......	10	177–205	189	2.8	4.7	......
Hybrids	......	......	......	2	......	......	......	......	......	......	......	2	239–244	241	......	......	46
P. jordani	......	......	......	......	......	......	6	4	......	......	......	10	285–316	301	3.0	3.15	......
C. c. sucklii	1	6	......	......	......	......	......	......	......	......	......	7	181–202	190	2.6	3.6	......
Hybrids	......	2*	2	3	......	......	......	......	......	......	......	7	187–232	215	7.0	8.6	25
P. d. delphinus	......	......	......	......	5	6	6	3	4	1	......	25	245–360	291	6.1	10.5	......
C. insignis	......	21	16	3	......	......	......	......	......	......	......	40	190–231	206	1.7	5.2	......
Hybrids	......	......	1	5	5	8	2	......	......	......	......	21	224–293	259	4.6	8.1	40
P. clarkii	......	......	......	......	......	......	3	5	19	10	3	40	288–374	338	3.0	5.6	......
Catostomus, sp.	3	1	......	......	......	......	......	......	......	......	......	4	168–195	177	6.0	5.9	......
Hybrids	......	1	20	21	2	......	......	......	......	......	......	44	200–251	226	1.7	5.0	47
P. santa-anae	......	......	......	1	6	20	16	3	......	......	......	46	244–324	282	2.5	6.0	......
C. tahoensis	7	11	4	......	......	......	......	......	......	......	......	22	166–218	191	2.8	6.9	......
Hybrids	......	......	1	8	1	......	......	......	......	......	......	10	212–254	233	3.5	4.75	40
P. lahontan	......	......	......	1	5	12	12	3	3	......	......	36	233–339	295	3.9	7.9	......
C. c. griseus	......	1	11	......	......	......	......	......	......	......	......	12	204–220	211	1.6	2.6	......
Hybrid	......	......	......	......	1	......	......	......	......	......	......	1	245	245	......	......	40
P. jordani	......	......	......	......	1	2	4	3	1	......	......	11	262–343	297	6.6	7.4	......
C. l. discobolus	......	1	4	......	......	......	......	......	......	......	......	5	194–221	210	4.5	4.8	......
Hybrid	......	......	......	1	......	......	......	......	......	......	......	1	231	231	......	......	23
P. d. utahensis	......	......	......	......	1	......	1	2	1	......	......	5	251–331	301	13.8	10.25	......
Totals:																	
Catostomus	15	46	36	3	......	......	......	......	......	......	......	100	166–231	200	1.5	7.5	......
Hybrids	......	3	24	40	9	8	2	......	......	......	......	86	187–293	235	2.2	8.8	42†
Pantosteus	......	......	......	2	18	40	48	23	28	11	3	173	233–374	302	2.4	15.4	......

* These low values for the character index are probably attributable to the small size of the 2 specimens (34 and 42.5 mm.)

† Grand average of all the individual hybrid indices.

the comparisons, we have united into 1 character index the 3 proportional measurements of the mouth that tend to be higher in *Pantosteus* than in *Catostomus,* namely, the over-all width, the width of the cartilaginous ridge of the mandible, and the length of the union of the lower lip; and for further distinctiveness of the index, we have added the proportionate width of the isthmus, which also tends to have a high value in *Pantosteus.* One reason for such a combination of proportionate measurements is to give joint expression to a series of characters and to minimize the importance of extreme fluctuations in single features. These objec~~tions~~s have been so well attained in the present application of the character index that it is possible to combine the indices for all localities and size groups of each combination without confusing the picture. Inspection of the frequency table indicates that the character indices of the hybrids overlap relatively little on those for the parental species, and a scrutiny of the raw data shows that perhaps half of this moderate overlap is attributable to age and local variation rather than to individual variation. Obviously, the hybrids have values for this index of mouth and isthmus dimensions that intervene between the usual figures for the parental species. Again the frequency distribution is unimodal, and apparently of essentially the normal type.

TABLE XII

FREQUENCY DISTRIBUTION OF THE HYBRID INDEX DERIVED FROM THE CHARACTER INDEX OF MOUTH AND ISTHMUS DIMENSIONS, FOR ALL *Catostomus* × *Pantosteus* HYBRIDS

Hybrid Index								Statistical Computations			
–5.1 to 5.0	5.1 to 15.0	15.1 to 25.0	25.1 to 35.0	35.1 to 45.0	45.1 to 55.0	55.1 to 65.0	65.1 to 75.0	No.	Range	Ave.	S.E.
1*	2*	9	4	32	24	11	3	86	–3 to 70	42	1.5

* The 2 very low values (–3 and 6) are due in part at least to the small size of the specimens (see footnote to Table XI); all other indices ranged from 14 to 70.

The relative growth of the lips and isthmus as combined into the character index seems to be intermediate in the hybrid: the index value increases with age in *Pantosteus clarkii,* remains nearly level in the hybrids, and decreases with age in *Catostomus insignis* (Fig. 4).

In this character index most of the hybrids, for some reason, approach the *Catostomus* parent somewhat more closely than they do the *Pantosteus* species involved in the cross. This relation is best brought out by computing, for each individual hybrid in each combination, the hybrid index (pp. 6–8), and then averaging the 86 indices—as done in Table XII. In each measurement involved in the computation of this hybrid index the hybrids tend to resemble the *Catostomus* parent the more closely; that is, the hybrid indices average lower than 50 (Table XXXVIII).

The species of *Pantosteus* usually have shorter dorsal fins, with fewer rays on the average, than do the forms of *Catostomus;* and the hybrids have intervening average numbers. This is indicated for 6 of the 7 combinations (Table XXXII). In the 1 exceptional case the parental types are very close, and there are only 2 hybrid specimens.

The interjacence of these intergeneric hybrids is well shown by the scale counts (Tables XXXIII to XXXVII). The hybrids tend to be intermediate in scale number whether the *Pantosteus* parent-form has fewer scales or more scales than the *Catostomus* species entering the cross.

It seems superfluous to compare all the intergeneric hybrids in detail with their respective parental species. There can be little doubt of the interpretations, except possibly for an occasional specimen. The hybrids show intermediacy not only in the characters in which the genera differ. They have values which interpose between those of the particular species of the 2 genera which occur in the same place.

Since 2 species of *Pantosteus* seldom if ever occur together, and since the species of *Catostomus* which here and there occur together are very distinct, we have felt little hesitancy in our interpretations of the species which are involved in each of the intergeneric crosses.

4. *Catostomus commersonnii sucklii* × *Pantosteus jordani*

(Pl. IV, Fig. 2)

U.M.M.Z. No. 120375: 1 half-grown 56 mm. long to caudal base, captured by L. C. Stuart and A. M. Stebler in Grace Coolidge Creek about 1 mile above State Game Lodge, Custer State Park, Custer County, South Dakota; August 7, 1934.

U.M.M.Z. No. 120381: 1 half-grown 57 mm. long, collected by L. C. Stuart in French Creek above the new dam about 5 miles east of Custer, Custer County, South Dakota; August 27, 1934.

TABLE XIII

RELATIVE NUMBERS OF HYBRIDS, *Catostomus commersonnii sucklii* × *Pantosteus jordani*, AND OF THE PARENTAL SPECIES

Specimens in University of Michigan Museum of Zoology	*Catostomus commersonnii sucklii*	Hybrids	*Pantosteus jordani*
In collection containing hybrid			
U.M.M.Z. No. 120375	14	1	37
U.M.M.Z. No. 120381	33	1	7
Total	47	2	44
From the Missouri River system	2111*	2	691

* Including a few specimens from the eastern part of the Missouri River system which may be referable to *C. c. commersonnii*. A considerable percentage of the *C. c. sucklii* total represents specimens from beyond the known range of *Pantosteus*.

These 2 sucker specimens can hardly represent any known or unknown species, for they are very distinctive in characters and come from the rather thoroughly collected region of the Black Hills. They obviously are hybrids

TABLE XIV

PROPORTIONATE MEASUREMENTS OF HYBRIDS BETWEEN *Catostomus commersonnii sucklii* AND *Pantosteus jordani*, AND OF THE PARENTAL SPECIES

The measurements are expressed as thousandths of the standard length. There are given for each item the range of the measurements; the average, in parentheses; and, as a subscript, the number of specimens.

French and Grace Coolidge Creeks, South Dakota			
Size group, mm.		Snout length	
C. commersonnii sucklii	50–70 $(62)_{10}$	*C. commersonni sucklii*	108–130 (121)
Hybrids	56–57 $(57)_{2}$	Hybrids	107–128 (117)
P. jordani	48–79 $(55)_{10}$	*P. jordani*	104–120 (110)
Caudal peduncle, depth		Mouth width	
C. commersonnii sucklii	85–94 (88)	*C. commersonnii sucklii*	74–86 (79)
Hybrids	85–89 (87)	Hybrids	94–102 (98)
P. jordani	78–91 (83)	*P. jordani*	102–115 (108)
Head length		Cartilaginous ridge, width	
C. commersonnii sucklii	284–301 (294)	*C. commersonnii sucklii*	36–44 (40)
Hybrids	267–281 (274)	Hybrids	47–48 (47)
P. jordani	243–265 (254)	*P. jordani*	62–69 (65)
Head depth		Mouth length	
C. commersonnii sucklii	168–182 (176)	*C. commersonnii sucklii*	53–62 (56)
Hybrids	165–167 (166)	Hybrids	65–68 (67)
P. jordani	143–160 (151)	*P. jordani*	63–78 (69)
Head width		Lower lip, union	
C. commersonnii sucklii	164–178 (169)	*C. commersonnii sucklii*	10–16 (14)
Hybrids	163–168 (165)	Hybrids	16–21 (19)
P. jordani	150–165 (158)	*P. jordani*	27–38 (32)
Isthmus width			
C. commersonnii sucklii	52–62 (55)		
Hybrids	73–82 (77)		
P. jordani	87–102 (96)		

between the 2 common species of the region. No other kind of either *Catostomus* or *Pantosteus* occurs there. They show intermediate values, or averages, for the:

Number of dorsal rays (Table XXXII),

Number of scales in all 5 series counted (Tables XXXIII to XXXVII),

Length, depth, and width of head; width of isthmus; length of snout; and the several mouth measurements (Table XIV),

Form of snout, structure and slant of mouth, coloration, etc. (as indicated on pp. 33–36; Pl. IV).

The average hybrid indices for the 2 specimens are respectively 39 and 48 (Table XXXIX).

5. *Catostomus commersonnii sucklii* × *Pantosteus delphinus delphinus*

(Pl. V, Fig. 2)

U.M.M.Z. No. 105638: 5 young and yearling specimens 34 to 74.5 mm. in standard length, seined by W. Petravicz and Middendorf in Colorado River, near its headwaters, at Hot Sulphur Springs, Grand County, Colorado; July 28, 1938.

U.M.M.Z. No. 136912: 1 half-grown 128 mm. and 1 adult 231 mm. long, collected by John Greenbank in Dry Creek, a tributary of Gunnison River, 3 miles west of Olathe, Montrose County, Colorado; April 21, 1941.

TABLE XV

Relative Numbers of Hybrids, *Catostomus commersonnii sucklii* × *Pantosteus delphinus delphinus*, and of the Parental Species

Specimens in University of Michigan Museum of Zoology	*Catostomus commersonnii sucklii*	Hybrids	*Pantosteus delphinus delphinus*
In collections containing hybrid			
U.M.M.Z. No. 105638	0	5	23
U.M.M.Z. No. 136912	1	2	11
Total	1	7	34
From the Colorado River system	10	7	2031*

* Many of these are from areas into which *C. c. sucklii* has not yet spread, or were collected prior to the introduction of that species.

The most exceptional and unexpected conditions under which these hybrids were obtained are explained on pp. 12–13. The very high frequency of the hybrids in comparison with the number of specimens of *Catostomus commersonnii sucklii* known from the Colorado River system is probably attributable to the recent introduction of that species into the Colorado River drainage. Such disruptions of long-standing ecological relationships apparently favor hybridization. The incidence of interspecific mating has presumably been increased by the scarcity of the introduced form and the abundance of the native *Pantosteus d. delphinus* at the times and places of collecting. The *sucklii* strays have probably found it as easy to contact spawning schools of *delphinus* as to establish pure spawning runs in an area already saturated with sucker populations.

The characters of these hybrids, at least on the average, are intercalated rather well between those of the parental species. This is true of the:

Number of dorsal rays (Table XXXII),
Number of scales in all 5 series counted (Tables XXXIII to XXXVII),
Depth of caudal peduncle (slender in *P. d. delphinus,* Pl. V), depth of head, width of isthmus, length of snout (in No. 105638 only), and in all dimensions of the mouth and lips (Table XVI). The length and the width of the head are the only noncommittal characters. Some of these characters are shown on the figures (Pl. V, Figs. 1–3).

It was first thought that the 2 specimens, No. 136912, might be hybrids between *Catostomus commersonnii sucklii* and *C. latipinnis discobolus,* but the measurements fitted that assumption very poorly, and a more detailed examination of the mouth disclosed *Pantosteus*-approaching features. The size of the scales further indicates that the called-for *Catostomus* parental species is neither the native *C. l. discobolus* nor *C. catostomus griseus,* which Mr. Greenbank also collected in the region. Particularly impressive is the approach shown by the hybrids to the peculiar squamational pattern of *P. d. delphinus,* which has very small scales forward but large ones in the caudal region (Pl. V).

Some of the 7 specimens approach the *Catostomus* parental species, others are closer to the *Pantosteus* form. The hybrid indices grade from 28 to 85, with an average of 53, close to the midpoint (Table XXXIX).

6. *Catostomus insignis* × *Pantosteus clarkii*

(Pl. I, Fig. 3*b*; Pl. VI, Fig. 3*b*)

U.M.M.Z. No. 110433: 1 half-grown 110 mm. in length to base of caudal fin, seined by C. M. Tarzwell in Taylor Creek, near the road between Beaverhead and Silver City, Gila National Forest, New Mexico; August 16, 1935.

U.M.M.Z. No. 118175: 3 young to half-grown 51 to 138 mm. long, taken by Gee and Carr of the U. S. Forest Service in Taylor Creek, New Mexico; August 26, 1937.

U.M.M.Z. No. 121642: 6 yearlings 73 to 94 mm. in length, seined by Tarzwell and Smith in the Middle Fork of the East Branch of Black River, at Crosby Ranch, Apache National Forest (Town 6 N., Range 28 E.), Arizona; July 22, 1937.

U.M.M.Z. No. 121646: 1 half-grown of 102 mm., taken by the same collectors in the West Fork of the East Branch of Black River at Three Forks, Apache National Forest (T. 5 N., R. 29 E.), Arizona; April 23, 1937.

U.M.M.Z. No. 125016: 3 yearlings 58 to 64 mm. long, collected by Carl L. Hubbs and family in Beaver Creek, near its junction with Verde River, near Camp Verde, Yavapai County (T. 14 N., R. 5 E.), Arizona; September 2, 1938.

U.M.M.Z. No. 125026: 7 young and yearling fish 47 to 76 mm. in length, obtained on the same day in Verde River, just above Camp Verde, near the mouth of Beaver Creek, Yavapai County, Arizona.

This is perhaps the most clear-cut and convincing of the hybrid combinations among the Catostomidae. In the region where hybrids were secured, the only sucker species usually present are *Catostomus insignis* and *Pantosteus clarkii,* and these species commingle in the same habitats. They are

TABLE XVI

PROPORTIONATE MEASUREMENTS OF HYBRIDS BETWEEN *Catostomus commersonnii sucklii* AND *Pantosteus delphinus delphinus*, AND OF THE PARENTAL SPECIES

The measurements are expressed as thousandths of the standard length. There are given for each item the range of the measurements; the average, in parentheses; and, as a subscript, the number of specimens.

	Hot Sulphur Springs, Colorado		Grand River System, Colorado	
Size group, mm.				
*C. c. sucklii**	32–57 $(44)_{10}$	66–125 $(98)_{7}$	…… $(155)_{1}$	189–239 $(213)_{8}$
Hybrids	34–43 $(40)_{3}$	57–71 $(64)_{2}$	…… $(128)_{1}$	…… $(231)_{1}$
P. d. delphinus	37–41 $(39)_{6}$	52–73 $(60)_{7}$	87–161 $(123)_{8}$	205–272 $(237)_{4}$
Caudal peduncle, depth				
C. c. sucklii	82–97 (93)	95–108 (98)	…… (97)	88–95 (91)
Hybrids	79–85 (82)	83–90 (87)	…… (84)	…… (90)
P. d. delphinus	73–79 (76)	75–83 (78)	68–76 (73)	70–74 (72)
Head length				
C. c. sucklii	279–305 (293)	264–293 (283)	…… (272)	238–276 (255)
Hybrids	252–284 (273)	256–285 (271)	…… (275)	…… (249)
P. d. delphinus	269–284 (277)	249–260 (256)	218–246 (234)	216–227 (222)
Head depth				
C. c. sucklii	171–182 (178)	155–181 (173)	…… (164)	156–167 (163)
Hybrids	156–165 (160)	156–169 (163)	…… (163)	…… (158)
P. d. delphinus	153–159 (155)	142–155 (149)	137–154 (144)	139–148 (145)
Head width				
C. c. sucklii	163–175 (168)	155–176 (166)	…… (166)	154–171 (164)
Hybrids	149–155 (152)	152–168 (160)	…… (171)	…… (168)
P. d. delphinus	154–163 (159)	153–167 (159)	158–170 (165)	168–175 (171)
Isthmus width				
C. c. sucklii	32–53 (42)	37–52 (47)	…… (50)	41–58 (49)
Hybrids	50–62 (55)	59–72 (65)	…… (66)	…… (56)
P. d. delphinus	77–103 (84)	63–87 (78)	79–104 (92)	98–135 (118)

* The figures entered for the *Catostomus* for Hot Sulphur Springs are transferred from Table II, which is based on material from the North Platte River of Wyoming and Nebraska, since none were secured at Hot Sulphur Springs and the species was probably introduced into the Upper Colorado River from the Platte River system.

TABLE XVI—(*Cont.*)

Proportionate Measurements of Hybrids between *Catostomus commersonnii sucklii* and *Pantosteus delphinus delphinus*, and of the Parental Species

	Hot Sulphur Springs, Colorado		Grand River System, Colorado	
Snout length				
C. c. sucklii	103–125 (114)	116–136 (126)	.... (139)	126–145 (133)
Hybrids	99–110 (105)	116–132 (124)	.... (146)	.... (142)
P. d. delphinus	93–105 (101)	105–122 (113)	111–136 (123)	124–145 (130)
Mouth width				
C. c. sucklii	72–85 (77)	67–90 (77)	.... (93)	82–88 (85)
Hybrids	77–83 (79)	88–103 (95)	.... (96)	.... (93)
P. d. delphinus	88–103 (95)	100–117 (110)	96–122 (111)	98–115 (105)
Cartilaginous ridge, width				
C. c. sucklii	40–48 (42)	39–53 (45)	.... (45)	39–42 (40)
Hybrids	46–50 (48)	55–56 (55)	.... (45)	.... (49)
P. d. delphinus	58–63 (60)	57–67 (62)	62–76 (69)	65–73 (69)
Mouth length				
C. c. sucklii	51–63 (56)	52–63 (56)	.... (65)	63–70 (67)
Hybrids	60–62 (61)	65–66 (65)	.... (72)	.... (72)
P. d. delphinus	54–66 (63)	63–76 (70)	68–84 (77)	75–84 (77)
Lower lip, union				
C. c. sucklii	7–18 (11)	13–18 (15)	.... (14)	13–18 (15)
Hybrids	14–19 (17)	17–19 (18)	.... (19)	.... (15)
P. d. delphinus	21–27 (24)	25–33 (27)	33–40 (36)	28–38 (34)

TABLE XVII

Relative Numbers of Hybrids, *Catostomus insignis* × *Pantosteus clarkii*, and of the Parental Species

Specimens in University of Michigan Museum of Zoology	*Catostomus insignis*	Hybrids	*Pantosteus clarkii*
In collection containing hybrid			
U.M.M.Z. No. 110433	32	1	18
U.M.M.Z. No. 118175	116	3	68
U.M.M.Z. No. 121642	14	6	36
U.M.M.Z. No. 121646	55	1	18
U.M.M.Z. No. 125016	96	3	168
U.M.M.Z. No. 125026	36	7	108
Total	349	21	416
From the Gila River system	1256	21	2069

strikingly different in a large number of characters, and the hybrids are consistently intermediate.

The dorsal rays average 11.00, a little less than midway between the mean of 11.32 for *C. insignis* and average of 10.59 for *P. clarkii* (Table XXXII).

The average number of the scales in this combination intervenes nicely between the means for the parental species (Tables XXXIII to XXXVII; Fig. 7). This is a particularly significant fact, in view of the very peculiar squamational pattern of *P. clarkii,* in which the scales are small except on the anterior part of the back, where they are spectacularly increased in size. In the series along the lateral line, below the lateral line, and around the caudal peduncle *clarkii* has finer scales than has *insignis;* but before the dorsal, and to a lesser degree in the row above the lateral line, the relation is reversed, in that *clarkii* here has fewer scales than does *insignis.* But in all series the hybrids are intermediate.

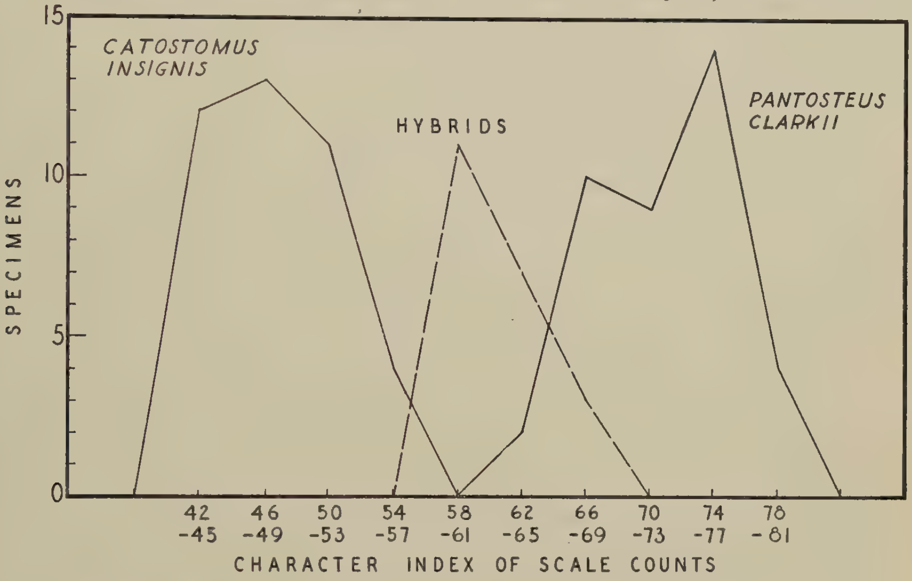

FIG. 5. Comparison of hybrids, *Catostomus insignis* × *Pantosteus clarkii,* with the parental species in the character index of scale counts (Table XVIII).

Much of the overlap between the scale counts of the hybrids and those of each parental form can be eliminated by combining all the enumerations of each specimen into a single index:

> (Scales in lateral line + scales below lateral line + scales around caudal peduncle) minus (scales before dorsal + scales above lateral line).

As shown in Table XVIII and in Figure 5 most of the indices for the hybrids are intercalated between the adjacent extremes of variation of the parental species. Thus, we again confirm the interpretation of the aberrant specimens as hybrids and verify the view that systematic characters do not mendelize in inheritance.

The hybrids display their origin and the mode of inheritance involved in almost all the proportionate measurements (Table XIX). Of the 10 measurements taken, only the head width fails to bear significantly on the problem. The length of the snout gives data that is hardly trustworthy, though the averages for the hybrids do lie between those for the parental species. Strikingly intermediate are the average values, in the hybrids, of the following proportionate measurements: depth of caudal peduncle, length and depth of head, width of isthmus, length and width of mouth, width of cartilaginous ridge of lower jaw, and length of union of lower lip.

TABLE XVIII

FREQUENCY DISTRIBUTION OF THE CHARACTER INDEX FOR SCALE COUNTS IN HYBRIDS, *Catostomus insignis* × *Pantosteus clarkii*, AND IN THE PARENTAL SPECIES

The index is derived by summing the counts of scales along lateral line, below lateral line, and around caudal peduncle; then subtracting from this sum the count of scales above the lateral line plus the predorsal scale count.

	Character Index for Scale Count									
	42–45	46–49	50–53	54–57	58–61	62–65	66–69	70–73	74–77	78–81
Catostomus insignis	12	13	11	4	......	......	......	......	......	......
Hybrids	......	......	......	......	11	7	3	......	......	......
Pantosteus clarkii	......	......	......	......	......	2	10	9	14	4

	Statistical Computations					
	No.	Range	Ave.*	S.E.*	V	Hybrid index $\pm$ S.E.$_I$
Catostomus insignis	40	42–55	48	.61	8.0	......
Hybrids	21	58–68	62	.69	5.1	58 ± 12
Pantosteus clarkii	39	65–81	72	.66	5.7	......

* Computed from the original, unclassed data.

In this combination the origin of the hybrids is particularly well shown by the structure of the mouth. The difference between the parental species involves all of the features mentioned in discussing the intergeneric hybrids (pp. 33–36). A very striking contrast is offered because the mouth of *P. clarkii* is especially extreme (Pl. I, Fig. 3*c*): it is extremely broad, and the gape almost strictly transverse; the lower lip is very full, and the median incision is shallow; the lateral emarginations are very deep. The mouths of the hybrids are invariably intermediate in structure (Pl. I, Figs. 3*a–c*) and obliquity (Pl. VI, Figs. 3*a–c*), and are almost always of intervening values in the dimensions (Fig. 4). Some specific features of *C. insignis*, such as the extreme turgidity of both lips and the fine, thick-set papillary pattern, are well reflected in the hybrids.

The character index for mouth and isthmus dimensions (Fig. 4) not only has intermediate values in the hybrids, but also indicates an intermediate relative growth for these dimensions. The index falls with age in *Catosto-*

TABLE XIX

PROPORTIONATE MEASUREMENTS OF HYBRIDS BETWEEN *Catostomus insignis* AND *Pantosteus clarkii*, AND OF THE PARENTAL SPECIES

The measurements are expressed as thousandths of the standard length. There are given for each item the range of the measurements; the average, in parentheses; and, as a subscript, the number of specimens.

	Verde River, Arizona	East Branch, Black River, Arizona		Taylor Creek, New Mexico	
Size group, mm.					
C. insignis	51–84 $(63)_{15}$	77–90 $(84)_{6}$	91–118 $(104)_{8}$	48–57 $(52)_{4}$	93–167 $(134)_{6}$
Hybrids	47–76 $(59)_{10}$	73–84 $(78)_{5}$	94–102 $(98)_{2}$	51–57 $(54)_{2}$	110–138 $(124)_{2}$
P. clarkii	49–92 $(61)_{15}$	58–78 $(64)_{9}$	91–179 $(125)_{6}$	49–82 $(62)_{6}$	127–163 $(146)_{4}$
Caudal peduncle, depth					
C. insignis	85–101 (94)	105–113 (109)	100–115 (107)	100–106 (103)	99–107 (102)
Hybrids	82–96 (88)	94–102 (98)	100–100 (100)	90–96 (93)	94–97 (95)
P. clarkii	76–87 (82)	93–103 (97)	83–98 (93)	78–88 (85)	80–90 (84)
Head length					
C. insignis	273–297 (284)	265–289 (278)	261–276 (271)	287–315 (302)	257–282 (267)
Hybrids	261–281 (273)	260–272 (267)	260–261 (261)	284–291 (277)	254–279 (267)
P. clarkii	245–272 (255)	255–281 (268)	224–267 (248)	237–274 (258)	232–257 (244)
Head depth					
C. insignis	172–193 (183)	186–195 (188)	172–190 (181)	189–204 (197)	170–188 (179)
Hybrids	165–180 (175)	172–180 (178)	164–176 (170)	184–185 (185)	162–172 (167)
P. clarkii	153–171 (163)	166–181 (173)	137–182 (161)	150–165 (160)	143–157 (151)
Head width					
C. insignis	162–191 (176)	158–192 (180)	166–187 (176)	185–191 (187)	168–182 (176)
Hybrids	162–176 (171)	179–185 (182)	168–183 (177)	176–186 (181)	173–179 (176)
P. clarkii	161–176 (169)	183–196 (187)	178–190 (184)	166–174 (170)	168–197 (182)
Isthmus width					
C. insignis	63–81 (70)	50–69 (61)	57–71 (63)	67–78 (70)	56–66 (64)
Hybrids	75–95 (84)	74–82 (78)	78–81 (79)	78–92 (85)	74–78 (76)
P. clarkii	93–115 (109)	104–121 (112)	99–111 (105)	101–120 (111)	94–113 (106)

TABLE XIX—(*Cont.*)

PROPORTIONATE MEASUREMENTS OF HYBRIDS BETWEEN *Catostomus insignis* AND *Pantosteus clarkii*, AND OF THE PARENTAL SPECIES

The measurements are expressed as thousandths of the standard length. There are given for each item the range of the measurements; the average, in parentheses; and, as a subscript, the number of specimens.

	Verde River, Arizona	East Branch, Black River, Arizona		Taylor Creek, New Mexico	
Snout length					
C. insignis	116–136 (129)	121–150 (141)	119–140 (133)	128–143 (136)	132–145 (138)
Hybrids	109–142 (121)	128–142 (134)	126–135 (131)	128–139 (133)	133–142 (137)
P. clarkii	108–135 (118)	125–141 (133)	120–135 (129)	122–138 (127)	127–145 (135)
Mouth width					
C. insignis	71–96 (81)	85–99 (93)	78–104 (89)	89–108 (97)	78–96 (85)
Hybrids	78–117 (92)	111–125 (117)	105–113 (109)	108–119 (113)	111–114 (113)
P. clarkii	90–126 (110)	125–137 (132)	127–152 (137)	116–137 (124)	120–143 (130)
Cartilaginous ridge, width					
C. insignis	38–52 (44)	37–43 (39)	31–41 (34)	37–51 (41)	29–41 (37)
Hybrids	48–67 (53)	44–53 (50)	50–56 (53)	55–55 (55)	55–55 (55)
P. clarkii	58–76 (67)	66–76 (70)	68–77 (71)	63–67 (66)	66–70 (68)
Mouth length					
C. insignis	53–76 (60)	49–72 (57)	50–72 (62)	67–73 (71)	66–82 (75)
Hybrids	52–87 (67)	76–89 (84)	74–83 (79)	85–87 (86)	85–86 (85)
P. clarkii	57–89 (71)	80–95 (88)	83–97 (91)	80–94 (87)	84–93 (87)
Lower lip, union					
C. insignis	9–14 (12)	12–20 (16)	12–19 (15)	9–20 (13)	12–18 (15)
Hybrids	12–24 (18)	21–27 (23)	25–27 (26)	19–27 (23)	25–29 (27)
P. clarkii	29–39 (35)	33–42 (36)	37–50 (43)	37–42 (39)	42–46 (43)

mus insignis, rises in *Pantosteus clarkii*, and remains about even in the hybrids.

As usual the hybrids are also intermediate in form of snout, in coloration, and in other characters (Pl. VI, Fig. 3). They tend to show their specific relationship to *C. insignis* in the development of dark spots on the scale pockets.

The intermediacy of the hybrids is beautifully shown by the limited deviation of the hybrid indices from the mean percentage value of 50 (Table

XXXIX). The total range is 25 to 67, and in 17 of the 21 hybrids the indices vary from 39 to 63.

7. *Catostomus,* species × *Pantosteus santa-anae*

(Fig. 6; Pl. I, Fig. 1*b*; Pl. VII, Fig. 1*b*)

U.M.M.Z. No. 131653: 1 young or yearling 45 mm. in standard length, seined by Robert R. and Ralph G. Miller in Santa Clara River 4.6 miles by road east of Piru, Ventura County, California; July 9, 1940.

U.M.M.Z. No. 131666: 5 adults 99 to 114 mm. long, taken by the same collectors in Sespe Creek, a tributary of Santa Clara River, 4.5 miles north-northwest of Fillmore, Ventura County, California; same date.

U.M.M.Z. No. 140484: 38 subadults and adults, 87–184 mm. long, secured by the same collectors in Sespe Creek, at base of dam about 0.5 mile above point where the collection was made in 1940; October 2, 1942.

TABLE XX

Relative Numbers of Hybrids, *Catostomus,* Species × *Pantosteus santa-anae,* and of the Parental Species

Specimens in University of Michigan Museum of Zoology	*Catostomus,* species	Hybrids	*Pantosteus santa-anae*
In collection containing hybrid			
U.M.M.Z. No. 131653	0	1	385
U.M.M.Z. No. 131666	2	5	207
U.M.M.Z. No. 140484	2	38	229
Total	4	44	825
From the Santa Clara System			
1934	......	......	355
1939	1	......	349
1940	2	6	845
1941	......	......	125
1942	2	38	234
Total	5	44	1907

The information on the collection of these hybrids and of the parental species poses a difficult problem. No species of sucker has been reported from the Santa Clara River system, though we have known for some years that this drainage is inhabited by *Pantosteus santa-anae* Snyder (1908)—the only species of the family hitherto known from the coastal streams of Southern California. Obviously one parent is this *Pantosteus,* which is locally abundant in the region where the hybrids were secured. The other parental species is obviously referable to *Catostomus,* but in the confused status of the taxonomy of this genus in the West Coast drainage it is inadvisable to attempt at present a more specific determination.

Since in 1939 a single specimen of this *Catostomus* was seined in Santa Clara River, a return trip was made to the region in 1940. Vigorous collecting in nearly all available habitats yielded only 2 more specimens (in Sespe Creek), along with hundreds of the *Pantosteus* and the 6 hybrids (1 from Santa Clara River; 5 from Sespe Creek).

In 1942 Sespe Creek was again visited. The seining was done in deep pools below a dam, for it was thought that the *Catostomus* might favor such a habitat. Only 2 specimens of this genus were obtained, however, along with the impressive number of 38 hybrids and 229 mountain suckers (*Pantosteus*). Of the *Pantosteus* 119 were discarded in the field after having been carefully checked by Robert R. Miller to make sure that no *Catostomus* and no hybrid was included. We are deeply grateful to Miller for his cooperation in this study. He obtained all of the hybrids of this combination.

Two explanations for the rarity of *Catostomus* in the Santa Clara River system offer themselves:

(1) It is a native species which was formerly common in the deeper holes but which is now approaching extinction because the surface waters are being consumed in irrigation and perhaps also because the holes in the river are being filled with flood washings. In years of more ample and more regular water supply this species and the *Pantosteus* may well have had a complementary distribution in the stream system; now they are forced to live together.

TABLE XXI

SEX RATIOS OF HYBRIDS BETWEEN *Catostomus*, SPECIES AND *Pantosteus santa-anae*, AND OF THE PARENTAL SPECIES

	Catostomus, species		Hybrids		*Pantosteus santa-anae*	
	Males	Females	Males	Females	Males	Females
Numbers	3	2	17	27	51	59*
Percentages	?	?	39	61	46	54*

* The slight preponderance of females in the *Pantosteus* sample, collected in 1942, may be explained by the facts that many of the suckers were discarded in the field, and that the largest ones, predominantly females, were preserved.

(2) It is an introduced species which has not become well established and which, like *Catostomus commersonnii sucklii* in the Colorado system (pp. 12–13 and 39), has been thrown out of adjustment with its environment. This alternative seems the less plausible, but is not readily to be excluded because we find very little basis, if any, for distinguishing the specimens taken in the Santa Clara from those collected in certain other watersheds in California.

Either theory offers conditions which, to judge from our studies on the hybridization of fish species in nature, would be conducive to the intermating of species and to the local extirpation of the rare parental form. The number of species of each category collected each year (Table XX) provides a slight indication that the population of the *Catostomus* has been encountered in the last stages of its decimation. It certainly seems probable that the number of hybrids has been increasing. In this connection it is of interest to note that the gonads of the hybrids and of the *Catostomus* specimens collected on October 2, 1942, showed evidence of little to no spawning during the previous breeding season. Most of the testes were large, and most of the ovaries were turgid with medium-sized eggs, not flabby and with relatively few eggs as in the *Pantosteus* specimens. The sex-ratio may be slightly altered in the hybrids (Table XXI).

The average values for the counts (Tables XXXII to XXXVII) are unusually close in the Santa Clara River forms of *Catostomus* and *Pantosteus* and hence offer a poorer test than usual of the behavior of characters in species crosses. A small difference between the species, with the hybrids intermediate on the average, is indicated by the counts of dorsal rays and of scales in the lateral line, in the predorsal region, and around the caudal peduncle. The number of scales in the transverse series (above and below the lateral line), however, averages a little higher in the hybrids than in either parental species. This unusual relation may represent a true condition. The greater number of scales in the transverse series may well be correlated with the greater depth of the body in the hybrids (Table XXII). In the minnow hybrid, *Gila orcuttii* × *Siphateles mohavensis,* a similar increase in transverse scale counts and in depth of body has been demonstrated and regarded as probably due to heterosis (Hubbs and Miller, 1943). The head averages longer than in either parental species, and the head and the caudal peduncle average about as deep and the dorsal fin about as large as in the *Catostomus;* the *Gila* × *Siphateles* hybrid is also large-headed and big-finned. In the head width, probably in the snout length, and certainly in the isthmus width and in all of the mouth measurements (which are particularly significant), the hybrids are intermediate between the *Catostomus* and the *Pantosteus* (some of these proportions are higher for the 1942 collections than for those of 1939 and 1940, due to the better preservation of the specimens). The interjacence of the hybrids is especially well shown by the character index which expresses the mouth and isthmus measurements (Table XI). The mouths of the hybrids show not only the usual features of intermediacy displayed by any of the *Catostomus* × *Pantosteus* crosses (pp. 33–36), but also present in diluted form the special peculiarities of each of the local parental species (Pl. I, Figs. 1*a–c;* Pl. VII, Figs. 1*a–c*).

The hybrid indices when summarized for individuals (Table XXXIX) fluctuate over a rather wide range, but tend toward an interjacent position. The mean value (41) is the lowest for any of the hybrid combinations. The variability and the low value are both caused by the peculiar relationships discussed in the preceding paragraph.

The intermediate hybrid nature of the aberrant suckers from the Santa Clara River system was confirmed by an analysis of an osteological character (Fig. 6), as represented in the 5 large specimens collected prior to 1942. The *Catostomus* species involved has a well-developed suboblong parietal fontanelle in the cranium, *Pantosteus santa-anae* typically has the fontanelle closed in the half-grown and adult, and the hybrids have an opening of

TABLE XXII

PROPORTIONATE MEASUREMENTS OF HYBRIDS BETWEEN *Catostomus*, SPECIES AND *Pantosteus santa-anae*, AND OF THE PARENTAL SPECIES

The measurements are expressed as thousandths of the standard length. There are given for each item the range of the measurements; the average, in parentheses; and, as a subscript, the number of specimens.

	Sespe Creek, 1942		Santa Clara River and Sespe Creek, 1939–40	Santa Clara River, 1940
Measured by	Hubbs		Johnson	Johnson
Size group, mm.				
Catostomus, sp.	....	92–109 (101)$_2$	120–153 (142)$_3$	....
Hybrids	141–184 (167)$_5$	87–130 (109)$_{33}$	99–114 (108)$_5$	.... (45)$_1$
P. santa-anae	....	93–123 (102)$_9$	89–126 (108)$_{22}$	39–49 (42)$_{15}$
Body depth				
Catostomus, sp.	....	227–251 (239)	204–252 (222)	....
Hybrids	224–239 (232)	223–268 (243)	219–233 (226)	.... (188)
P. santa-anae	....	214–238 (226)	173–263 (197)	188–214 (201)
Caudal peduncle, depth				
Catostomus, sp.	....	93–104 (99)	86–98 (92)	....
Hybrids	97–101 (99)	91–104 (98)	96–98 (97)	.... (97)
P. santa-anae	....	86–97 (90)	78–92 (85)	89–98 (93)
Head length				
Catostomus, sp.	....	243–268 (255)	226–247 (236)	....
Hybrids	256–265 (263)	247–277 (264)	251–263 (256)	.... (272)
P. santa-anae	....	254–269 (263)	231–264 (249)	266–286 (278)
Head depth				
Catostomus, sp.	....	163–174 (169)	144–152 (149)	....
Hybrids	162–173 (168)	154–184 (169)	158–170 (162)	.... (160)
P. santa-anae	....	155–171 (164)	136–159 (149)	161–170 (166)
Head width				
Catostomus, sp.	....	161–172 (167)	148–159 (153)	....
Hybrids	172–190 (181)	168–187 (177)	158–163 (161)	.... (156)
P. santa-anae	....	175–188 (182)	152–175 (161)	153–170 (164)

TABLE XXII—(*Cont.*)

	Sespe Creek, 1942		Santa Clara River and Sespe Creek, 1939–40	Santa Clara River, 1940
Measured by	Hubbs		Johnson	Johnson
Snout length				
Catostomus, sp.	....	112–113 (113)	107–120 (113)	....
Hybrids	120–131 (127)	109–130 (122)	113–134 (126)	.... (125)
P. santa-anae	....	123–139 (132)	105–132 (118)	107–126 (117)
Mouth width				
Catostomus, sp.	....	79–82 (81)	78–82 (80)	....
Hybrids	90–101 (94)	89–110 (99)	95–104 (99)	.... (104)
P. santa-anae	....	119–134 (127)	94–117 (107)	111–126 (117)
Cartilaginous ridge, width				
Catostomus, sp.	....	39–44 (41)	35–43 (39)	....
Hybrids	48–54 (51)	44–56 (51)	42–51 (47)	.... (47)
P. santa-anae	....	59–68 (64)	47–61 (54)	52–62 (57)
Mouth length				
Catostomus, sp.	....	51–54 (53)	49–57 (54)	....
Hybrids	60–71 (64)	59–79 (69)	62–72 (69)	.... (71)
P. santa-anae	....	84–95 (90)	66–80 (73)	71–85 (79)
Lower lip, union				
Catostomus, sp.	....	15–22 (19)	14–17 (15)	....
Hybrids	21–29 (25)	20–29 (25)	15–22 (19)	.... (19)
P. santa-anae	....	37–48 (42)	28–40 (35)	28–37 (33)
Isthmus width				
Catostomus, sp.	....	.... (64)*	33–55 (45)	....
Hybrids	79–87 (81)	63–94 (81)	56–74 (66)	.... (64)
P. santa-anae	....	83–116 (98)	56–98 (78)	57–91 (80)

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* In the smaller of the two hybrids in this set the gill membranes form a free fold across the isthmus, as in the teratological suckers described by Hubbs (1914*a*). In this specimen the scales are not increased in number as they were in the terata previously described; on the contrary the lateral line count is extremely low (69). The isthmus measurement of this aberrant fish is not included.

TABLE XXII—(*Cont.*)

Proportionate Measurements of Hybrids between *Catostomus*, Species and *Pantosteus santa-anae*, and of the Parental Species

	Sespe Creek, 1942		Santa Clara River and Sespe Creek, 1939–40	Santa Clara River, 1940
Measured by	Hubbs		Johnson	Johnson
Dorsal fin to occiput, distance				
Catostomus, sp.	....	285–288 (287)	256–274 (266)	....
Hybrids	281–300 (292)	274–301 (288)	240–261 (252)	.... (267)
P. santa-anae	....	269–298 (285)	242–284 (270)	239–263 (252)
Dorsal fin base				
Catostomus, sp.	....	154–182 (168)	153–180 (168)	....
Hybrids	171–188 (183)	157–188 (172)	162–179 (167)	.... (180)
P. santa-anae	....	147–196 (167)	133–164 (147)	142–160 (151)
Depressed dorsal fin, length				
Catostomus, sp.	....	233–301 (267)	233–292 (268)	....
Hybrids	254–280 (267)	241–300 (267)	253–283 (268)	.... (271)
P. santa-anae	....	233–286 (255)	207–254 (225)	235–255 (247)

TABLE XXIII

Dimensions of the Parietal Fontanelle in Hybrids between *Catostomus*, Species and *Pantosteus santa-anae*, and in the Parental Species

	Standard length, mm. (L)	Length of Fontanelle	Width of Fontanelle	Area, mm² (A)	Relative Area $\left(\frac{100\sqrt{A}}{L}\right)$
Catostomus, species	119 152 153	6.51 8.30 7.80	1.70 1.49 1.90	9.48 9.99 11.70	2.59 2.08 1.90
Hybrids	99 108 109 110 114	5.16 4.85 5.86 5.32 5.39	0.89 1.20 0.97 1.00 1.23	3.47 2.85 3.53 3.70 3.35	1.88 1.56 1.72 1.75 1.61
Pantosteus santa-anae	99 107 109.5 114 115 117.5 126	3.12 1.90 0.00 0.00 0.00 0.00 0.00	0.20 0.19 0.00 0.00 0.00 0.00 0.00	0.35 0.25 0.00 0.00 0.00 0.00 0.00	0.60 0.00 0.49 0.00 0.00 0.00 0.00
Average					
Catostomus, species	141	7.54	1.70	10.39	2.30
Hybrids	108	5.32	1.06	3.38	1.70
Pantosteus	113	0.72	0.06	0.08	0.15

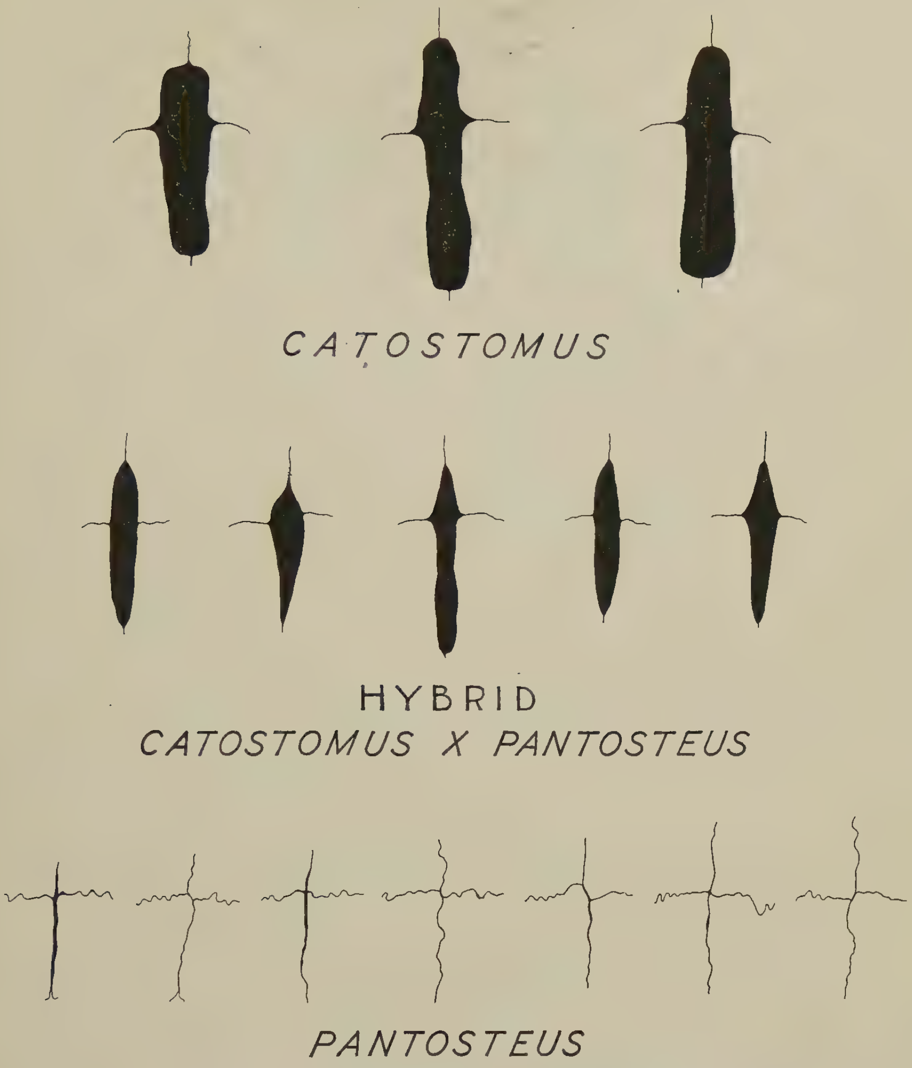

FIG. 6. Comparison of parietal fontanelles in hybrids, *Catostomus,* species × *Pantosteus santa-anae,* and in the parental species (Table XXIII).

All figures were drawn to the same scale with the aid of a camera lucida.

Top row, *Catostomus,* species: left, from specimen 120 mm. long, taken in Santa Clara River, Ventura County, California; center and right, from specimens respectively 152 and 153 mm. long, seined with the hybrids.

Middle row, hybrids: from right to left, drawn from 5 specimens grading from 99 to 114 mm. in length (U.M.M.Z. No. 131666).

Bottom row, *Pantosteus santa-anae:* sketched from 7 specimens, grading from 99 mm. (at left) to 126 mm. long.

intermediate size. The fontanelle in the hybrids tends to be pointed fore and aft. The length and width figures as well as the area computations are given in Table XXIII. The area was obtained by proportion from (1) the length of the fontanelle as measured on the specimen to .01 mm., with precision dial calipers; (2) the length of the opening as measured on the figure, which was carefully drawn with the aid of a camera lucida; (3) the area of the sketch of the fontanelle, determined by planimeter. The hybrid indices average 33 for the length, 39 for the width, and 28 for the relative area of the fontanelle. The closer approach toward the *Catostomus* thus indicated may be due to the facts that the fontanelle tends to become reduced in relative size with age, and that the measured hybrids average smaller than the 3 specimens of the *Catostomus* which were examined for this character.

8. *Catostomus tahoensis* × *Pantosteus lahontan*

(Pl. I, Fig. 2*b*; Pl. VII, Fig. 2*b*)

U.M.M.Z. No. 124861: 3 half-grown measuring 71 to 84 mm. in standard length, collected by Carl L. Hubbs and family in Carson River, 2 miles west of Fallon, Nevada, at an elevation of 3970 feet; July 30, 1938.

U.M.M.Z. No. 124870: 1 half-grown 96 mm. long, obtained on the same expedition in Humboldt River at Irish-American Dam, about 3 miles northeast of Lovelock, Nevada; July 31, 1938.

U.M.M.Z. No. 124919: 3 young to small adult fish 35.5 to 128 mm. long, also taken on the same trip, in Hot Creek, tributary to Pony Creek and thence to Pine Creek, in the Humboldt River system in Eureka County, 31.6 miles by road south of Carlin, Nevada; August 12, 1938.

U.M.M.Z. No. 133688: a breeding pair 106 and 122 mm. long, collected by Garth Murphy in Little Truckee River just above the head of Boca Dam, about 7 miles east of Truckee, Nevada County, California; June 26, 1940.

U.M.M.Z. No. 133816: 1 half-grown 86 mm. in length, taken by J. R. Alcorn in Carson River at Coleman Dam, Churchill County, Nevada; April 27, 1940.

U.M.M.Z. No. 136273: 1 yearling 46 mm. long, seined by Carl L. Hubbs and family in North Fork of Humboldt River near mouth, Elko County, Nevada, June 29, 1942.

U.M.M.Z. No. 136274: 1 adult of 123.5 mm., taken on the same trip in Truckee River, 3.3 miles above Wadsworth, Washoe County, Nevada, July 22, 1942.

U.M.M.Z. No. 140235: 6 yearling to small adult specimens 67–118 mm. long, seined by Robert R. Miller and party in Little Truckee River, 14 miles north of Truckee, Sierra County, California; August 15, 1942.

U.M.M.Z. No. 140243: 2 yearlings 44 to 55 mm. long, taken on the same expedition in Prosser Creek, 4.5 miles north of Truckee, Nevada County, California; August 15, 1942.

U.M.M.Z. No. 140285: 3 young and 1 small adult 24 to 96 mm. long, secured on the same trip in a distributary of the East Fork of Carson River, Douglas County, Nevada; August 19, 1942.

U.M.M.Z. No. 140302: 2 adults 157 to 165 mm. long, obtained by the same party in Carson River, 12 miles east of Carson City, Nevada; August 20, 1942.

U.M.M.Z. No. 140370: 1 half-grown of 80 mm., seined on the same trip in a distributary of Buckeye Creek, about 3 miles north of Bridgeport, Mono County, California; August 26, 1942.

U.M.M.Z. No. 140390: a 68 mm. yearling, also taken on the same expedition in a distributary of Buckeye Creek, below Highway 395; August 28, 1942.

These hybrids between the 2 common suckers of the Lahontan interior drainage basin conform to the general pattern. They exhibit intermediacy in the various ways that all *Catostomus* × *Pantosteus* do (pp. 33–36).

In number of dorsal rays (Table XXXII) the hybrids yield an intermediate average despite the fact that the means for the parental species differ by only 0.28 of a ray. Though the parental differences in the scale counts are not large, intermediacy of the averages is indicated for the 10 specimens collected prior to 1942. Except for fin-ray counts no enumerations or measurements were taken on the 18 obvious hybrids collected in 1942, when the manuscript was in press.

TABLE XXIV

RELATIVE NUMBERS OF HYBRIDS, *Catostomus tahoensis* × *Pantosteus lahontan*, AND OF THE PARENTAL SPECIES

Specimens in University of Michigan Museum of Zoology	*Catostomus tahoensis*	Hybrids	*Pantosteus lahontan*
In collection containing hybrid			
U.M.M.Z. No. 124861	88	3	39
U.M.M.Z. No. 124870	13	1	177
U.M.M.Z. No. 124919	7	3	70
U.M.M.Z. No. 133688	2	2	16
U.M.M.Z. No. 133816	1	1	1
U.M.M.Z. No. 136273	12	1	46
U.M.M.Z. No. 136274	85	1	5
U.M.M.Z. No. 140235	33	6	31
U.M.M.Z. No. 140243	10	2	49
U.M.M.Z. No. 140285	50	4	181
U.M.M.Z. No. 140302	58	2	57
U.M.M.Z. No. 140370	80	1	4
U.M.M.Z. No. 140390	51	1	1
Total	500	28	677
From the Lahontan system	3282*	28	2007

* Perhaps one-third of the *Catostomus* specimens were collected in places where neither the *Pantosteus* nor hybrids occur. *Pantosteus* is very generally accompanied by *Catostomus.*

In either 2 or all 3 of the series as tabulated, representing either 9 or 10 of the 10 specimens, the average values for the following proportionate measurements of the hybrids measured (Table XXV) intervene between those of the parental species: length, depth, and width of the head, the width of the isthmus, and all the mouth measurements. The length of the snout shows nothing important, and there is some doubt as to the significance of the head-width proportion. The form of the snout, as well as that of the mouth, however, bespeaks the origin of the hybrid (Pl. I, Figs. 2*a*–*c*; Pl. VII, Figs. 2*a*–*c*).

The 10 individual hybrid indices based on all counts and measurements vary from 15 to 86, and average 44, not far below the theoretically perfect intermedial value of 50 (Table XXXIX).

TABLE XXV

Proportionate Measurements of Hybrids between *Catostomus tahoensis* and *Pantosteus lahontan*, and of the Parental Species

The measurements are expressed as thousandths of the standard length. There are given for each item the range of the measurements; the average, in parentheses; and, as a subscript, the number of specimens.

	Carson and Humboldt Rivers and Hot Creek, Nevada		Little Truckee River, California
Size group, mm.			
C. tahoensis	32–43 (37)$_8$	62–89 (76)$_{12}$	77–134 (105)$_2$
Hybrids	.... (35)$_1$	71–128 (89)$_7$	106–122 (114)$_2$
P. lahontan	31–53 (35)$_{10}$	63–99 (80)$_{20}$	87–108 (97)$_6$
Caudal peduncle, depth			
C. tahoensis	75–84 (79)	86–95 (89)	90–95 (93)
Hybrids	.... (86)	83–96 (88)	95–96 (96)
P. lahontan	75–86 (81)	72–92 (84)	86–92 (90)
Head length			
C. tahoensis	260–294 (275)	246–275 (257)	248–261 (255)
Hybrids	.... (283)	241–255 (246)	242–242 (242)
P. lahontan	240–279 (264)	217–245 (233)	226–239 (235)
Head depth			
C. tahoensis	164–183 (171)	147–164 (157)	152–158 (155)
Hybrids	.... (160)	136–151 (147)	150–151 (151)
P. lahontan	141–158 (152)	122–142 (136)	129–142 (137)
Head width			
C. tahoensis	156–165 (160)	146–167 (154)	150–153 (151)
Hybrids	.... (152)	148–164 (152)	150–158 (154)
P. lahontan	147–155 (150)	137–156 (149)	149–169 (158)
Isthmus width			
C. tahoensis	52–66 (59)	57–71 (63)	52–62 (57)
Hybrids	.... (89)	68–85 (73)	75–75 (75)
P. lahontan	77–103 (89)	86–103 (94)	83–99 (91)

TABLE XXV—(*Cont.*)

PROPORTIONATE MEASUREMENTS OF HYBRIDS BETWEEN *Catostomus tahoensis* AND *Pantosteus lahontan*, AND OF THE PARENTAL SPECIES

The measurements are expressed as thousandths of the standard length. There are given for each item the range of the measurements; the average, in parentheses; and, as a subscript, the number of specimens.

	Carson and Humboldt Rivers and Hot Creek, Nevada		Little Truckee River, California
Snout length			
C. tahoensis	89–109 (97)	98–130 (111)	116–117 (117)
Hybrids	.... (102)	104–123 (112)	115–121 (118)
P. lahontan	93–110 (104)	102–117 (110)	109–129 (122)
Mouth width			
C. tahoensis	70–87 (78)	73–94 (78)	79–81 (80)
Hybrids	.... (87)	79–100 (89)	89–89 (89)
P. lahontan	74–99 (91)	91–114 (102)	93–126 (116)
Cartilaginous ridge, width			
C. tahoensis	32–47 (39)	38–46 (41)	36–39 (37)
Hybrids	.... (48)	45–50 (48)	46–49 (47)
P. lahontan	43–59 (55)	51–61 (57)	61–72 (68)
Mouth length			
C. tahoensis	44–61 (52)	46–64 (52)	53–55 (54)
Hybrids	.... (59)	57–72 (63)	60–62 (61)
P. lahontan	55–74 (65)	63–75 (69)	70–91 (80)
Lower lip, union			
C. tahoensis	9–15 (12)	10–13 (12)	13–14 (13)
Hybrids	.... (17)	20–25 (22)	20–24 (22)
P. lahontan	19–36 (25)	27–41 (35)	37–46 (41)

After the foregoing account had been prepared it was discovered that *Catostomus tahoensis* and *Pantosteus lahontan* differ in the usual number of pelvic rays, and that in this character the hybrids are almost exactly intermediate (Table XXVI).

Intermediacy of the hybrids in developmental features of pigmentation was observed in studying the 1942 collections. As the scales become developed anteriorly the scale pockets become rather sharply outlined with dark in *Pantosteus lahontan,* but are only obscurely bordered in *Catostomus tahoensis.* At this stage the pigment on the side of the head barely extends to the line passing through the lower border of the eye in the *Pantosteus,* but tends to pass below that point in the *Catostomus.* As development proceeds the lower border of the lateral band on the head is gradually extended below the eye in the *Pantosteus,* but at comparable stages the pigment extends farther down in the *Catostomus.* Furthermore, the lower margin of the pigment tends to form an even and regular line in the *Pantosteus,* so that the head appears sharply bicolored, whereas in the *Catostomus* the lower border of the pigment is less even and the lower side of the face tends to be mottled (Pl. VII, Figs. 2*a–c*). In all these respects the hybrids are intermediate.

TABLE XXVI

NUMBER OF PELVIC RAYS IN HYBRIDS BETWEEN *Catostomus tahoensis* AND *Pantosteus lahontan,* AND IN THE PARENTAL SPECIES*

	Number of Rays in Both Pelvic Fins								
	16	17	18	19	20	21	22	23	24
C. tahoensis	......	......	6	30	269	63	77	1	1
Hybrids	......	......	9	5	13	1	......	......	......
P. lahontan	12	17	297	24	29	......	......	......	......

	Statistical computations					
	No.	Range	Ave.	S.E.	V	Hybrid Index ± S.E.$_{\mathrm{I}}$
C. tahoensis	447	18–24	20.41	.04	4.5	....
Hybrids	28	18–21	19.21	.18	4.9	52 ± 9
P. lahontan	379	16–20	18.11	.04	4.0	....

* These data were obtained too late to be used in the subsequent computations of hybrid intermediacy.

9. *Catostomus catostomus griseus* × *Pantosteus jordani*

(Pl. VII, Fig. 3*b*)

U.M.M.Z. No. 127541: 1 half-grown 98 mm. in the standard length measurement, collected by Carl L. and Laura C. Hubbs in Sweetwater River at Hudson's Ranch, Fremont County (Town 30 N., Range 95 W.), Wyoming; July 11, 1934.

TABLE XXVII

RELATIVE NUMBERS OF HYBRIDS, *Catostomus catostomus griseus* × *Pantosteus jordani,* AND OF THE PARENTAL SPECIES

Specimens in University of Michigan Museum of Zoology	*Catostomus catostomus griseus*	Hybrids	*Pantosteus jordani*
In collection containing hybrid			
U.M.M.Z. No. 127541	2	1	55
U.M.M.Z. No. 127548	13	1	1
Total	15	2	56
From the Platte River system	692*	2	81

* Some of these specimens came from localities below the known altitudinal range of *Pantosteus jordani.*

TABLE XXVIII

PROPORTIONATE MEASUREMENTS OF HYBRIDS BETWEEN *Catostomus catostomus griseus* AND *Pantosteus jordani*, AND OF THE PARENTAL SPECIES

The measurements are expressed as thousandths of the standard length. There are given for each item the range of the measurements; the average, in parentheses; and, as a subscript, the number of specimens.

Sweetwater River and Alkali Creek, Wyoming

Size group, mm.		Snout length	
C. c. griseus	79–173 $(118)_{12}$	*C. c. griseus*	105–135 (117)
Hybrid	$(98)_{1}$	Hybrid	(115)
P. jordani	83–125 $(98)_{10}$	*P. jordani*	105–112 (109)
Caudal peduncle, depth		Mouth width	
C. c. griseus	79–95 (86)	*C. c. griseus*	84–93 (88)
Hybrid	(93)	Hybrid	(93)
P. jordani	81–88 (85)	*P. jordani*	94–110 (102)
Head length		Cartilaginous ridge, width	
C. c. griseus	241–266 (258)	*C. c. griseus*	43–48 (46)
Hybrid	(235)	Hybrid	(52)
P. jordani	210–232 (220)	*P. jordani*	61–70 (66)
Head depth		Mouth length	
C. c. griseus	138–153 (148)	*C. c. griseus*	59–70 (66)
Hybrid	(136)	Hybrid	(64)
P. jordani	122–138 (129)	*P. jordani*	60–72 (66)
Head width		Lower lip, union	
C. c. griseus	155–171 (161)	*C. c. griseus*	10–17 (13)
Hybrid	(152)	Hybrid	(27)
P. jordani	143–150 (147)	*P. jordani*	30–41 (37)
Isthus width			
C. c. griseus	60–71 (64)		
Hybrid	(73)		
P. jordani	85–102 (92)		

U.M.M.Z. No. 127548: 1 yearling seined by Carl L. Hubbs and family in Alkali Creek, a tributary of Sweetwater River, 14 miles by road from U. S. Highway 87W toward the Red Desert, Fremont County (T. 29 N., R. 96 W.), Wyoming; July 11, 1934.

Since hybrid No. 127548 became misplaced before detailed counts and measurements were taken, nothing more can be said of it than that it was compared with No. 127541 and found similar, that it was also intermediate between the assigned parental species, and that an identification of the hybrid as between *Catostomus commersonnii sucklii* and *Pantosteus jordani* seemed excluded by such characters as a high scale count. *C. c. sucklii* was collected with the hybrids (44 with No. 127541 and 24 with No. 127548).

There can be no doubt that 1 parent of these 2 hybrids was *Pantosteus jordani* (if that is the proper name for the form occupying Sweetwater River). That the other parent was *C. catostomus griseus* rather than *C. commersonii sucklii* seems evident from the high scale counts (Tables XXXIII–XXXVII; Fig. 7). No. 127541 shows scale counts that intervene nicely between the means for *griseus* and *jordani.* The dorsal ray count (Table XXXII) is inconclusive.

In its proportionate measurements (Table XXVIII) the single hybrid at hand is intermediate between *griseus* and *jordani* in the length, depth, and width of the head, the width of the isthmus, the length of the snout, the over-all width of the mouth, the width of the cartilaginous ridge, and the length of the union of the lower lips. It is off-center in respect to the depth of the caudal peduncle and the length of the mouth (virtually insignificant characters in this comparison).

The hybridity of the available example is confirmed by the various features shared in general by the *Catostomus* × *Pantosteus* hybrids (pp. 33–36; Pl. VII, Figs. 3*a*–*c*). Its hybrid index is 44.

10. *Catostomus latipinnis discobolus* × *Pantosteus delphinus utahensis*

U.M.M.Z. No. 124767: 1 young 25 mm. long, collected by Carl L. Hubbs and family just above the mouth of a small tributary of Virgin River, immediately below mouth of La Verkin Creek, near La Verkin (Town 41 S., Range 13 W.), Utah; July 3, 1938.

TABLE XXIX

Relative Numbers of Hybrids, *Catostomus latipinnis discobolus* × *Pantosteus delphinus utahensis*, and of the Parental Species

Specimens in University of Michigan Museum of Zoology	*Catostomus latipinnis discobolus*	Hybrid	*Pantosteus delphinus utahensis*
In collection containing hybrid U.M.M.Z. No. 124767	16	1	119
From the Virgin River system	125	1	590

This additional combination between a fine-scaled *Catostomus* and a *Pantosteus* falls in line with the other interspecific sucker hybrids in regard to the relationship between its characters and those of the parental species. The scales appear to be intermediate in number, but are too poorly developed

TABLE XXX

PROPORTIONATE MEASUREMENTS OF HYBRIDS BETWEEN *Catostomus latipinnis discobolus* AND *Pantosteus delphinus utahensis*, AND OF THE PARENTAL SPECIES

The measurements are expressed as thousandths of the standard length. There are given for each item the range of the measurements; the average, in parentheses; and, as a subscript, the number of specimens.

	Tributary of Virgin River, Utah	
Size group, mm.		
C. l. discobolus	...... $(35)_1$	89–105 $(97)_4$
Hybrid	...... $(25)_1$	....
P. d. utahensis	30–35 $(33)_4$	...... $(89)_1$
Caudal peduncle, depth		
C. l. discobolus	...... (60)	58–63 (61)
Hybrid	...... (80)	....
P. d. utahensis	75–84 (79)	...... (80)
Head length		
C. l. discobolus	...... (279)	254–272 (265)
Hybrid	...... (287)	....
P. d. utahensis	281–299 (289)	...... (248)
Head depth		
C. l. discobolus	...... (168)	157–165 (160)
Hybrid	...... (168)	....
P. d. utahensis	160–171 (168)	...... (146)
Head width		
C. l. discobolus	...... (151)	162–172 (165)
Hybrid	...... (162)	....
P. d. utahensis	154–169 (167)	...... (162)
Isthmus width		
C. l. discobolus	...... (54)	53–57 (55)
Hybrid	...... (78)	....
P. d. utahensis	83–103 (92)	...... (101)
Snout length		
C. l. discobolus	...... (105)	137–144 (139)
Hybrid	...... (107)	....
P. d. utahensis	105–123 (117)	...... (107)

TABLE XXX—(*Cont.*)

PROPORTIONATE MEASUREMENTS OF HYBRIDS BETWEEN *Catostomus latipinnis discobolus* AND *Pantosteus delphinus utahensis,* AND OF THE PARENTAL SPECIES

The measurements are expressed as thousandths of the standard length. There are given for each item the range of the measurements; the average, in parentheses; and, as a subscript, the number of specimens.

	Tributary of Virgin River, Utah	
Mouth width		
C. l. discobolus	.... (89)	100–113 (105)
Hybrid	.... (86)	....
P. d. utahensis	91–124 (113)	.... (127)
Cartilaginous ridge, width		
C. l. discobolus	.... (38)	36–39 (37)
Hybrid	.... (48)	....
P. d. utahensis	53–62 (58)	.... (64)
Mouth length		
C. l. discobolus	.... (64)	82–88 (85)
Hybrid	.... (59)	....
P. d. utahensis	64–84 (75)	.... (91)
Lower lip, union		
C. l. discobolus	.... (13)	11–20 (16)
Hybrid	.... (19)	....
P. d. utahensis	24–31 (30)	.... (39)

in the one small example to be counted with accuracy. The dorsal rays (Table XXXII) number 11 instead of usually 13 or 14, as in the local stock of *C. l. discobolus,* or 9 or 10, as in *P. d. utahensis.*

Material for a comparison of the proportionate measurements (Table XXX) is very unsatisfactory. The most critical measurements, of the width of the isthmus and of the cartilaginous ridge, and of the length of the union of the lower lips, do exhibit the expected intermediacy. Under the circumstances the over-all width of the mouth does not. The other measurements are hardly critical enough to warrant consideration.

CONCLUSIONS

Although we miss the evidence of experimental matings and of cytological abnormalities that is available for other families of fishes, we feel that the data presented in this paper demonstrate that distinct species of the fish family Catostomidae do hybridize in nature. In general, the natural hybrids in this family seem to be of rather rare and sporadic occurrence (Table XXXI). The 182 hybrids identified in this family constitute 11.5 and 7.2 per cent of the specimens, respectively, of the more primitive and of the more specialized of the parental species with which they happened to be collected, but only 1.8 and 1.5 per cent of the specimens of the 2 species from the same river systems (as delimited in Table XXXI). If we could satisfactorily exclude the specimens that were taken in those parts of the river systems that are inhabited by only 1 of the species, the ratio of hybrid frequency would be raised somewhat. In those parts of the stated stream systems where both parental species occur, perhaps 1 sucker in 100 on the average is an int~~ra~~erspecific hybrid.

In 6 of the 10 crosses the frequency of hybridization is probably less than the average figures just given, but in 4 of the combinations, the hybrids seem to occur much more commonly. The hybrids between the not closely related species *Catostomus macrocheilus* and *C. syncheilus* (both subspecies) constitute 15.1 and 21.6 per cent of the individuals of the respective parental forms with which they were collected, and 6.4 and 4.2 per cent of the total numbers of the parental species at hand from the same river systems. In 2 combinations the hybrids are scarce in comparison with one of the parental species but, on the basis of the known material, actually seem in one cross to approximate and in the other to outnumber the second parental species, which is extremely rare in the same river system. There has obviously been in each case some extreme disruption of the normal ecological relationships. In one of the combinations we can feel reasonably sure that the basis for the anomalous mating relationship was the recent introduction of one of the forms, *Catostomus commersonnii sucklii,* into the Colorado River system, where *Pantosteus d. delphinus,* the other parental species, abounds. In the other similar situation, we can explain the extreme rarity of one parental species (*Catostomus,* species) as due either to its recent introduction, or, more plausibly, to the destruction of its former habitat, following the use of the stream water for irrigation. On either basis, this rare form would have been forced into association with an abundance of another species, *Pantosteus santa-anae,* with which most of its few spawners would appear to have hybridized. In this situation the rare species appears from observation as well as on theoretical grounds to be approaching extinction. The 2 specimens of *Catostomus* and the 38 hybrids collected on October 2, 1942, showed gonadal evidence of having spawned little to not at all during the preceding spawning season. In this combination the sex ratio may be somewhat disturbed.

The rare and sporadic occurrence of specimens unclassifiable as to species, except where the normal population ratios have been greatly disturbed as just indicated, is a main reason for the interpretation of the aberrant individuals as hybrids. The presumed parental species are known to occur in the same system and have generally been collected with the fish thought

TABLE XXXI

SUMMARY DATA ON RELATIVE NUMBERS OF CATOSTOMID HYBRIDS AND OF THE PARENTAL KINDS

Derived from Tables I, IV, IX, XIII, XV, XVII, XX, XXIV, XXVII, and XXIX. See these tables for further details and for qualifying remarks.

Hybrid Combination	River System	More Primitive Parental Species (Coarse-scaled *Catostomus* in *Catostomus* × *Catostomus* Crosses, or Any *Catostomus* in the *Catostomus* × *Pantosteus* Combinations)		Hybrids	More Specialized Parental Species (Fine-scaled *Catastomus* in *Catostomus* × *Catostomus* Crosses, or *Pantosteus* Species in the *Catostomus* × *Pantosteus* Combinations)	
		Collected in Same River System	Collected with Hybrids		Collected with Hybrids	Collected in Same River System
Catostomus commersonnii sucklii *Catostomus catostomus griseus*	Platte	1250	169	5	41	692
Catostomus macrocheilus *Catostomus syncheilus syncheilus*	Columbia (Palouse excepted)	969	384	69	273	1341*
Catostomus macrocheilus *Catostomus syncheilus palouseanus*	Palouse (Washington)	152	93	3	60	358
Catostomus commersonnii sucklii *Pantosteus jordani*	Missouri	2111†	47	2	44	691
Catostomus commersonnii sucklii *Pantosteus delphinus delphinus*	Colorado	10	1	7	34	2031
Catostomus insignis *Pantosteus clarkii*	Gila (Arizona)	1256	349	21	416	2069
Catostomus, species *Pantosteus santa-anae*	Santa Clara (California)	5	4	44	825	1907
Catostomus tahoensis *Pantosteus lahontan*	Lahontan	3282	500	28	677	2007
Catostomus catostomus griseus *Pantosteus jordani*	Platte	692	15	2	56	81
Catostomus latipinnis discobolus *Pantosteus delphinus utahensis*	Virgin (Utah, etc.)	125	16	1	119	590
Totals	All named	9852†	1578	182	2545	11767‡

* Including some specimens probably referable to other subspecies.

† Including the 1250 specimens of *Catostomus commersonnii sucklii* from the Platte River System, enumerated also in the count for the Missouri System.

‡ Including the 692 specimens of *Catostomus catostomus griseus*, enumerated also as the more primitive species in another combination.

to be hybrids. No integrity of populations or of spawning runs, or other evidence of specific integrity, has been indicated for the aberrant types. Their characters, particularly in the intergeneric crosses, *Catostomus* × *Pantosteus,* are discordant with the taxonomic system of the family. Nor do they have features which would lead one to regard them as extreme variants or mutant sports of any recognized species. No assumption other than that of the intermating of species in nature agrees with the facts.

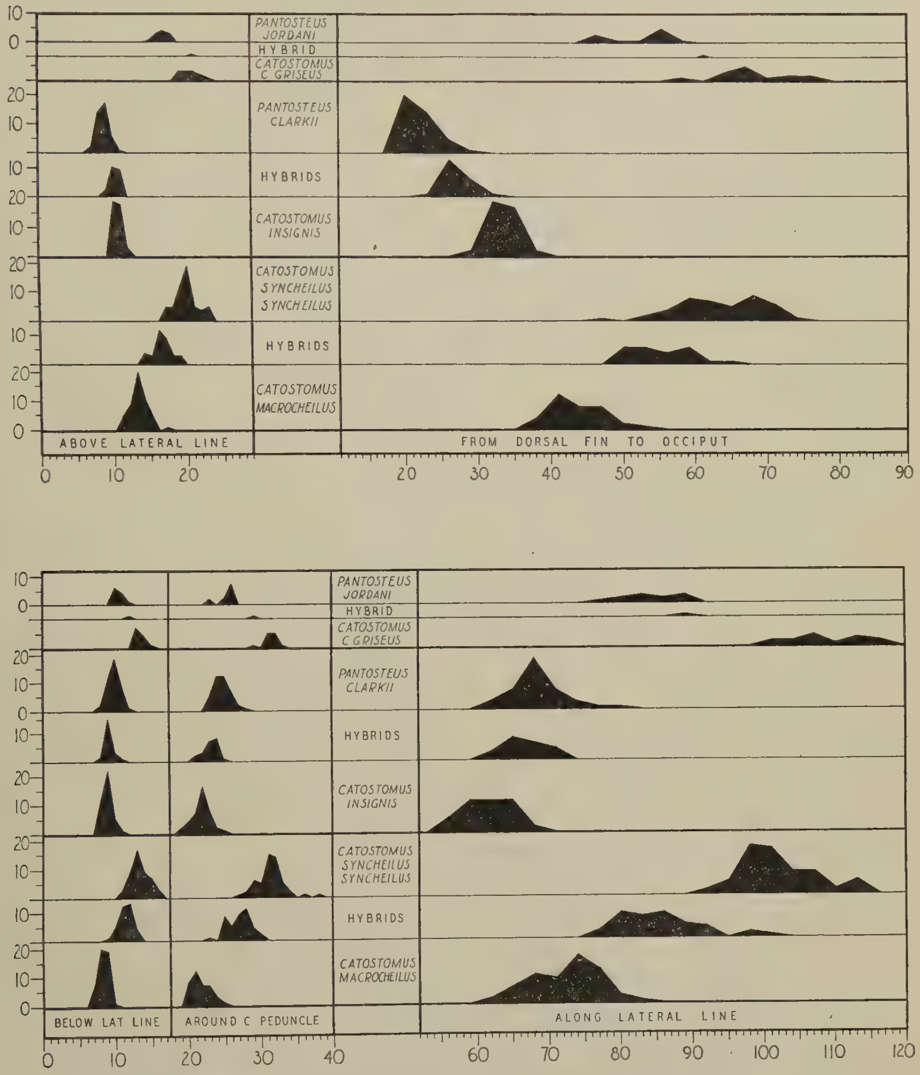

FIG. 7. Comparison of various sucker hybrids with their parental species in the 5 scale counts (Tables XXXIII–XXXVII).

The most cogent reason that favors the hypothesis of hybridity is that

TABLE XXXII

NUMBER OF DORSAL RAYS IN SUCKER HYBRIDS AND IN PARENTAL SPECIES

	Number of Dorsal Rays								Statistical Computations					
	9	10	11	12	13	14	15	16	No.	Range	Ave.	S.E.	V	Hybrid Index
Catostomus commersonnii sucklii	......	......	23	7	......	......	......	......	30	11–12	11.23	.08	3.9	......
Hybrids	......	3	2	......	......	......	......	......	5	10–11	10.40	.24	5.2	69
Catostomus catostomus griseus	1	33	......	1	......	......	......	......	35	9–12	10.03	.06	3.5	......
Catostomus macrocheilus	......	......	......	2	24	37	12	1	76	12–16	13.82	.09	5.7	......
Hybrids	......	......	1	27	34	6	......	......	68	11–14	12.66	.08	5.2	64
Catostomus syncheilus syncheilus	......	......	17	46	17	......	......	......	80	11–13	12.00	.07	5.2	......
Catostomus macrocheilus	......	......	......	6	21	23	......	......	50	12–14	13.34	.10	5.3	......
Hybrids	......	......	......	2	1	......	......	......	3	12–13	12.33	.33	4.6	46
Catostomus syncheilus palouseanus	......	3	30	9	......	......	......	......	42	10–12	11.14	.08	4.65	......
Catostomus commersonnii sucklii	......	......	9	1	......	......	......	......	10	11–12	11.10	.10	2.85	......
Hybrids	......	1	1	......	......	......	......	......	2	10–11	10.50?		......	86?
Pantosteus jordani	......	6	4	......	......	......	......	......	10	10–11	10.40	.16	4.9	......
*Catostomus commersonnii sucklii**	......	......	29	8	......	......	......	......	37	11–12	11.22	.07	3.8	......
Hybrids	......	3	4	......	......	......	......	......	7	10–11	10.57	.14	3.5	88
Pantosteus delphinus delphinus	......	13	12	......	......	......	......	......	25	10–11	10.48	.10	4.8	......
Catostomus insignis	......	......	27	13	......	......	......	......	40	11–12	11.33	.07	3.9	......
Hybrids	......	3	15	3	......	......	......	......	21	10–12	11.00	.12	5.0	45
Pantosteus clarkii	......	16	23	......	......	......	......	......	39	10–11	10.59	.08	4.7	......
Catostomus, species	......	3	2	......	......	......	......	......	5	10–11	10.40	.24	4.6	......
Hybrids	2	30	12	......	......	......	......	......	44	9–11	10.23	.08	5.2	40
Pantosteus santa-anae†	13	190	9	......	......	......	......	......	212	9–11	9.98	.02	2.9	......
Catostomus tahoensis	1	165	95	4	......	......	......	......	265	9–12	10.38	.03	5.05	......
Hybrids	......	20	7	1	......	......	......	......	28	10–12	10.32	.10	5.2	21
Pantosteus lahontan	3	186	25	......	......	......	......	......	214	9–11	10.10	.02	3.4	......
Catostomus catostomus griseus	4	8	......	......	......	......	......	......	12	9–10	9.67	.14	5.0	......
Hybrid	......	1	......	......	......	......	......	......	1	10	10.00?			137?
Pantosteus jordani	2	8	1	......	......	......	......	......	11	9–11	9.91	.16	5.4	......
Catostomus latipinnis discobolus	......	......	......	......	3	2	......	......	5	13–14	13.40	.24	4.0	......
Hybrid	......	......	1	......	......	......	......	......	1	11	11.00?			63?
Pantosteus delphinus utahensis	2	3	......	......	......	......	......	......	5	9–10	9.60	.24	5.6	......

* Including the counts listed in the top row.

† Of the 212 counts, 168 were furnished by Robert R. Miller.

TABLE XXXIII

Number of Lateral Line Scales in Sucker Hybrids and in Parental Species

	Number of Lateral Line Scales (Frequencies)																								Statistical Computations					
	55–57	58–60	61–63	64–66	67–69	70–72	73–75	76–78	79–81	82–84	85–87	88–90	91–93	94–96	97–99	100–102	103–105	106–108	109–111	112–114	115–117	118–120	121–123	124–126	No.	Range*	Ave.*	S.E.*	V*	Hybri Inde
C. commersonnii sucklii	9	12	2	4	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	27	55–65	59.11	.59	5.2	......
Hybrids	......	......	......	......	......	1	1	2	......	......	1	......	......	......	......	......	......	......	......	......	......	......	......	......	5	72–87	77.00	2.51	7.3	42
C. catostomus griseus	......	......	......	......	......	......	......	......	......	......	3	2	4	1	3	1	4	3	2	5	2	1	......	......	31	86–118	102.16	1.83	10.0	......
C. macrocheilus	......	......	2	6	10	9	12	7	3	1	......	......	......	......	......	......	......	......	......	......	......	......	......	......	50	61–83	71.66	.70	6.9	......
Hybrids	......	......	......	......	......	......	......	3	9	8	9	5	4	......	2	1	......	......	......	......	......	......	......	......	41	76–100	85.34	.90	6.75	45
C. syncheilus syncheilus	......	......	......	......	......	......	......	......	......	......	......	......	1	5	17	16	8	8	2	5	......	......	......	......	62	93–113	101.97	.65	5.0	......
C. macrocheilus	......	......	......	......	4	4	16	9	3	......	2	......	1	......	......	......	......	......	......	......	......	......	......	......	39	67–93	75.31	.79	6.55	......
Hybrids	......	......	......	......	......	......	......	......	......	......	1	......	......	......	1	......	......	......	......	......	......	......	......	......	2	85–99	92.0?	......	......	56
C. syncheilus palouseanus	......	......	......	......	......	......	......	......	......	......	......	1	......	1	2	7	11	7	7	1	1	......	......	1	39	90–124	105.23	.96	5.7	......
C. commersonnii sucklii	2	2	5	1	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	10	56–64	60.50	.92	10.0	......
Hybrids	......	......	......	......	1	......	......	1	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	2	67–77	72.5?	......	......	45
P. jordani	......	......	......	......	......	......	......	2	......	2	1	2	1	1	1	......	......	......	......	......	......	......	......	......	10	78–97	86.90	2.11	7.7	......
C. commersonnii sucklii†	9	13	4	6	2	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	34	55–68	60.12	.59	5.7	......
Hybrids	......	......	1	......	......	1	1	......	......	1	......	1	......	......	1	1	......	......	......	......	......	......	......	......	7	63–100	82.29	5.43	17.5	52
P. delphinus delphinus	......	......	......	......	......	......	......	......	......	1	......	1	......	2	4	3	3	8	2	......	......	1	......	......	25	84–118	102.36	1.43	7.0	......
C. insignis	5	11	11	11	2	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	40	56–67	61.55	.53	5.45	......
Hybrids	......	......	3	8	6	4	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	21	61–70	66.57	.64	4.4	72
P. clarkii	......	......	3	7	18	7	3	1	1	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	40	61–79	68.53	.60	5.5	......
Catostomus, species	......	......	......	......	1	......	1	......	......	1	1	1	......	......	......	......	......	......	......	......	......	......	......	......	5	69–88	80.40	3.61	9.0	......
Hybrids	......	......	......	1	......	1	9	11	9	9	3	1	......	......	......	......	......	......	......	......	......	......	......	......	44	66–89	78.57	.68	5.8	73
P. santa-anae	......	......	......	......	1	......	10	13	17	4	1	......	......	......	......	......	......	......	......	......	......	......	......	......	46	69–87	77.89	.47	4.1	......
C. tahoensis	......	......	......	......	......	......	......	......	1	2	2	3	5	......	......	......	......	......	......	......	......	......	......	......	13	80–93	88.08	1.15	8.8	......
Hybrids	......	......	......	......	......	......	1	1	......	3	......	1	3	......	......	......	......	......	......	......	......	......	......	......	9	73–92	85.15	2.05	7.2	60
P. lahontan	......	......	......	......	......	1	......	6	6	4	3	3	2	1	1	......	......	......	......	......	......	......	......	......	27	72–99	83.22	1.24	7.7	......
C. catostomus griseus	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	1	1	4	1	3	2	......	......	......	12	102–117	109.50	1.37	4.3	......
Hybrid	......	......	......	......	......	......	......	......	......	......	......	1	......	......	......	......	......	......	......	......	......	......	......	......	1	90	90.0?	......	......	77
P. jordani	......	......	......	......	......	......	......	1	2	3	2	3	......	......	......	......	......	......	......	......	......	......	......	......	11	77–89	84.09	1.23	4.85	......

* Computed from the original, unclassed data.

† Including the counts listed in the top row.

TABLE XXXIV

NUMBER OF PREDORSAL SCALES IN SUCKER HYBRIDS AND IN PARENTAL SPECIES

	Number of Predorsal Scales (Frequencies)																				Statistical Computations					
	19–21	22–24	25–27	28–30	31–33	34–36	37–39	40–42	43–45	46–48	49–51	52–54	55–57	58–60	61–63	64–66	67–69	70–72	73–75	76–78	No.	Range*	Ave.*	S.E.*	V*	Hybrid Index
C. commersonnii sucklii	......	......	......	3	13	6	3	......	......	......	......	......	......	......	......	......	......	......	......	......	25	28–39	33.08	.52	7.9	......
Hybrids	......	......	......	......	......	......	......	......	1	1	1	......	......	......	......	......	......	......	......	......	3	45–49	47.00	1.73	6.4	52
C. catostomus griseus	......	......	......	......	......	......	......	......	......	4	1	1	3	6	4	8	2	......	1	......	30	46–73	59.70	1.27	11.65	—
C. macrocheilus	......	......	......	......	......	......	4	12	8	8	2	1	......	......	......	......	......	......	......	......	35	37–53	43.57	.63	8.55	......
Hybrids	......	......	......	......	......	......	......	......	......	......	6	6	4	6	1	1	......	......	......	......	24	49–64	55.13	.87	7.7	59
C. syncheilus syncheilus	......	......	......	......	......	......	......	......	......	1	......	2	4	8	7	5	9	6	1	......	43	46–75	63.26	.92	9.5	......
C. macrocheilus	......	......	......	......	......	1	2	10	11	5	......	......	......	......	......	......	......	......	......	......	29	36–48	42.76	.55	6.9	......
Hybrids	......	......	......	......	......	......	......	......	......	......	......	......	1	......	......	1	......	......	......	......	2	56–65	60.5?	......	......	82?
C. syncheilus palouseanus	......	......	......	......	......	......	......	......	......	......	......	......	1	4	9	7	4	4	1	......	30	56–74	64.50	.80	6.8	—
C. commersonnii sucklii	......	......	......	......	1	7	2	......	......	......	......	......	......	......	......	......	......	......	......	......	10	33–38	35.30	.54	4.8	......
Hybrids	......	......	......	......	......	......	......	1	......	1	......	......	......	......	......	......	......	......	......	......	2	40–47	45.0?	..	..	50?
P. jordani	......	......	......	......	......	......	......	......	......	1	1	3	2	2	1	......	......	......	......	......	10	48–61	54.80	1.43	8.25	—
C. commersonnii sucklii†	......	......	......	3	13	13	3	......	......	......	......	......	......	......	......	......	......	......	......	......	32	28–39	33.47	.46	7.8	......
Hybrids	......	......	......	......	......	......	1	1	......	1	1	......	1	......	......	......	......	......	......	......	5	39–57	46.40	3.28	15.8	50
P. delphinus delphinus	......	......	......	......	......	......	......	......	......	......	1	2	4	4	4	5	......	......	......	......	20	49–66	59.45	.99	7.45	......
C. insignis	......	......	......	2	19	17	2	......	......	......	......	......	......	......	......	......	......	......	......	......	40	30–37	33.43	.32	6.05	......
Hybrids	......	1	13	6	1	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	21	24–31	27.00	.43	7.3	56
P. clarkii	20	14	5	1	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	40	19–28	22.03	.38	10.9	......
Catostomus, species	......	......	......	......	......	......	......	2	3	......	......	......	......	......	......	......	......	......	......	......	5	40–45	42.80	.86	4.0	......
Hybrids	......	......	......	......	......	......	7	24	12	1	......	......	......	......	......	......	......	......	......	......	44	38–46	41.55	.29	4.7	35
P. santa-anae	......	......	......	......	......	8	14	19	5	......	......	......	......	......	......	......	......	......	......	......	46	34–44	39.24	.39	6.7	—
C. tahoensis	......	......	......	......	......	......	......	1	1	4	5	2	1	......	......	......	......	......	......	......	14	42–55	48.93	1.02	7.8	......
Hybrids	......	......	......	......	......	......	......	......	3	3	1	......	1	1	......	......	......	......	......	......	9	44–58	48.56	1.68	10.4	116
P. lahontan	......	......	......	......	......	......	......	......	6	10	5	6	......	1	......	......	......	......	......	......	28	43–58	48.61	.72	7.8	—
C. catostomus griseus	......	......	......	......	......	......	......	......	......	......	......	......	......	1	......	3	5	1	1	1	12	60–76	68.00	1.33	6.8	......
Hybrid	......	......	......	......	......	......	......	......	......	......	......	......	......	......	1	......	......	......	......	......	1	61	61.0?	......	......	47?
P. jordani	......	......	......	......	......	......	......	......	......	3	1	1	5	1	......	......	......	......	......	......	11	46–59	53.00	1.34	8.4	—

* Computed from original, unclassed data.

† Including the counts listed in the top row.

TABLE XXXV

NUMBER OF SCALES ABOVE THE LATERAL LINE IN SUCKER HYBRIDS AND IN PARENTAL SPECIES

	Number of Scales above the Lateral Line (Frequencies)																					Statistical Computations					
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	No.	Range	Ave.	S.E.	V	Hybrid Index
C. commersonnii sucklii	......	......	5	15	6	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	26	9–11	10.04	.13	6.5	......
Hybrids	......	......	......	......	......	1	1	......	......	1	1	......	......	......	......	......	......	......	......	......	......	4	12–17	14.50	1.19	16.4	52
C. catostomus griseus	......	......	......	......	......	......	......	......	......	4	2	7	7	6	1	2	......	......	......	......	......	29	16–22	18.69	.31	8.9	......
C. macrocheilus	......	......	......	......	5	9	20	11	5	......	1	......	......	......	......	......	......	......	......	......	......	51	11–17	13.12	.17	9.25	......
Hybrids	......	......	......	......	......	......	......	4	3	12	9	3	3	......	......	......	......	......	......	......	......	34	14–19	16.38	.24	8.5	48
C. syncheilus syncheilus	......	......	......	......	......	......	......	......	......	......	5	5	11	19	5	4	5	......	......	......	......	54	17–23	19.85	.22	8.1	......
C. macrocheilus	......	......	......	......	2	5	16	13	2	1	1	......	......	......	......	......	......	......	......	......	......	40	11–17	13.37	.18	8.5	......
Hybrids	......	......	......	......	......	......	......	......	......	......	......	1	1	......	......	......	......	......	......	......	......	2	18–19	18.5?	......	......	62?
C. syncheilus palouseanus	......	......	......	......	......	......	......	......	......	1	......	1	2	5	11	5	6	2	3	......	1	37	16–27	21.65	.35	9.8	......
C. commersonnii sucklii	......	......	2	5	3	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	10	9–11	10.10	.23	7.2	......
Hybrids	......	......	......	......	......	......	2	......	......	......	......	......	......	......	......	......	......	......	......	......	......	2	13–13	13.0?	......	......	38?
P. jordani	......	......	......	......	......	......	......	......	......	2	2	3	2	1	......	......	......	......	......	......	......	10	16–20	17.80	.42	7.5	......
*C. commersonnii sucklii**	......	......	5	19	9	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	33	9–11	10.12	.11	6.3	......
Hybrids	......	......	......	......	3	......	......	1	2	1	......	......	......	......	......	......	......	......	......	......	......	7	11–16	13.29	.84	16.7	62
P. delphinus delphinus	......	......	......	......	......	......	......	......	......	2	5	2	3	3	4	1	......	......	......	......	......	20	16–22	18.80	.41	9.75	......
C. insignis	......	......	......	19	18	3	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	40	10–12	10.60	.10	6.0	......
Hybrids	......	......	2	10	9	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	21	9–11	10.33	.14	6.2	15
P. clarkii	2	14	17	6	1	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	40	7–11	8.75	.14	10.1	......
Catostomus, species	......	......	......	......	......	......	1	......	2	2	......	......	......	......	......	......	......	......	......	......	......	5	13–16	15.00	.58	7.7	......
Hybrids	......	......	......	......	......	......	......	7	19	13	5	......	......	......	......	......	......	......	......	......	......	44	14–17	15.36	.13	5.7	−34
P. santa-anae	......	......	......	......	......	......	17	17	10	2	......	......	......	......	......	......	......	......	......	......	......	46	13–16	13.93	.09	4.2	......
C. tahoensis	......	......	......	......	......	......	......	......	1	4	4	3	2	......	......	......	......	......	......	......	......	14	15–19	17.07	.32	7.0	......
Hybrids	......	......	......	......	......	......	......	......	......	4	4	1	......	......	......	......	......	......	......	......	......	9	16–18	16.67	.24	4.3	23
P. lahontan	......	......	......	......	......	......	4	4	4	11	4	1	......	......	......	......	......	......	......	......	......	28	13–18	15.36	.26	9.0	......
C. catostomus griseus	......	......	......	......	......	......	......	......	......	......	......	......	3	3	3	2	1	......	......	......	......	12	19–23	20.58	.37	6.2	......
Hybrid	......	......	......	......	......	......	......	......	......	......	......	......	......	......	1	......	......	......	......	......	......	1	21	21.0?	......	......	−11?
P. jordani	......	......	......	......	......	......	......	......	1	3	4	3	......	......	......	......	......	......	......	......	......	11	15–18	16.82	.30	5.9	......

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* Including the counts listed in the top row.

TABLE XXXVI

Number of Scales below the Lateral Line in Sucker Hybrids and in Parental Species

	Scales below Lateral Line (Frequencies)											Statistical Computations					
	7	8	9	10	11	12	13	14	15	16	17	No.	Range	Ave.	S.E.	V	Hybrid Index
C. commersonnii sucklii	15	11	1	......	......	......	......	......	......	......	......	27	7–9	7.48	.11	7.6	......
Hybrids	......	1	......	1	2	......	......	......	......	......	......	4	8–11	10.00	.71	14.2	63
C. catostomus griseus	......	......	2	7	8	10	4	......	2	......	......	33	9–15	11.45	.25	12.5	......
C. macrocheilus	8	20	19	1	......	......	......	......	......	......	......	48	7–10	8.27	.11	9.2	......
Hybrids	......	......	1	5	12	13	4	......	......	......	......	35	9–13	11.40	.16	8.3	62
C. syncheilus syncheilus	......	......	......	......	3	9	17	9	7	3	......	48	11–16	13.35	.19	9.9	......
C. macrocheilus	1	9	23	5	2	......	......	......	......	......	......	40	7–11	8.95	.13	9.2	......
Hybrids	......	1	......	......	2	......	......	......	......	......	......	3	8–11	10.0?	1.00	17.3?	19?
C. syncheilus palouseanus	......	......	......	......	1	2	2	15	11	7	3	41	11–17	14.61	.22	9.6	......
C. commersonnii sucklii	4	6	......	......	......	......	......	......	......	......	......	10	7–8	7.60	.16	6.7	......
Hybrids	......	2	......	......	......	......	......	......	......	......	......	2	8–8	8.0?	......	......	12?
P. jordani	......	......	1	1	5	3	......	......	......	......	......	10	9–12	11.00	.30	9.6	......
*C. commersonnii sucklii**	16	17	1	......	......	......	......	......	......	......	......	34	7–9	7.56	.10	7.7	......
Hybrids	......	1	2	......	3	1	......	......	......	......	......	7	8–12	10.14	.55	14.35	49
P. delphinus delphinus	......	......	......	5	2	2	2	3	3	3	......	20	10–16	12.85	.50	17.4	......
C. insignis	......	12	22	5	1	......	......	......	......	......	......	40	8–11	8.88	.11	7.8	......
Hybrids	......	2	15	3	1	......	......	......	......	......	......	21	8–11	9.14	.14	7.0	26
P. clarkii	......	2	11	18	8	1	......	......	......	......	......	40	8–12	9.88	.14	9.0	......
Catostomus, species	......	......	......	......	1	3	1	......	......	......	......	5	11–13	12.00	.32	5.3	......
Hybrids	......	......	......	......	3	9	16	12	3	1	......	44	11–16	13.14	.17	8.4	139
P. santa-anae	......	......	......	......	4	11	9	5	4	......	......	33	11–15	12.82	.21	9.3	......
C. tahoensis	......	......	......	4	4	7	3	2	......	......	......	20	10–14	11.75	.28	10.7	......
Hybrids	......	......	1	3	4	1	1	......	......	......	......	10	9–13	10.80	.34	9.95	52
P. lahontan	......	2	7	2	1	3	1	......	......	......	......	16	8–13	9.94	.19	7.65	......
C. catostomus griseus	......	......	......	......	......	......	7	4	1	......	......	12	13–15	13.50	.18	4.6	......
Hybrid	......	......	......	......	......	1	......	......	......	......	......	1	12	12.0?	......	......	51?
P. jordani	......	......	......	6	4	1	......	......	......	......	......	11	10–12	10.55	.20	6.3	......

* Including the counts listed in the top row.

TABLE XXXVII

NUMBER OF SCALES AROUND THE CAUDAL PEDUNCLE IN SUCKER HYBRIDS AND IN PARENTAL SPECIES

	Number of Scales around Caudal Peduncle (Frequencies)																					Statistical Computations					
	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	No.	Range	Ave.	S.E.	V	Hybrid Index
C. commersonnii sucklii	......	7	11	5	2	2	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	27	19–23	20.30	.23	5.9	......
Hybrids	......	......	......	......	......	......	......	......	......	1	2	1	......	......	......	......	......	......	......	......	......	4	27–29	28.00	.41	2.9	72
C. catostomus griseus	......	......	......	......	......	......	......	......	1	1	3	4	3	6	8	4	1	1	1	......	......	33	26–36	30.97	.39	7.2	......
C. macrocheilus	3	4	15	13	9	3	1	......	......	......	......	......	......	......	......	......	......	......	......	......	......	48	18–24	20.71	.19	6.4	......
Hybrids	......	......	......	......	......	1	......	8	5	9	11	4	2	......	......	......	......	......	......	......	......	40	23–30	27.00	.25	5.9	59
C. syncheilus syncheilus	......	......	......	......	......	......	......	......	......	1	2	6	5	15	14	5	2	......	1	......	1	52	27–38	31.29	.26	6.0	......
C. macrocheilus	......	......	8	12	7	7	3	1	......	......	......	......	......	......	......	......	......	......	......	......	......	38	20–25	21.68	.22	6.25	......
Hybrids	......	......	......	......	......	......	......	......	1	......	1	......	......	......	......	......	......	......	......	......	......	2	26–28	27.0?	......	......	49?
C. syncheilus palouseanus	......	......	......	......	......	......	......	......	......	......	......	......	7	10	5	6	4	4	1	2	1	40	30–38	32.55	.35	6.8	......
C. commersonnii sucklii	1	3	2	3	1	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	10	18–22	20.00	.39	6.2	......
Hybrids	......	......	......	......	......	......	1	1	......	......	......	......	......	......	......	......	......	......	......	......	......	2	24–25	24.5?	......	......	58?
P. jordani	......	......	......	......	......	......	......	......	2	2	3	3	......	......	......	......	......	......	......	......	......	10	26–29	27.70	.37	4.2	......
*C. commersonnii sucklii**	1	7	17	5	2	2	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	34	18–23	20.18	.19	5.5	......
Hybrids	......	......	......	......	1	2	......	......	......	2	1	......	1	......	......	......	......	......	......	......	......	7	22–30	25.71	1.14	11.7	64
P. delphinus delphinus	......	......	......	......	......	......	......	1	1	5	3	6	1	3	3	......	......	......	......	......	......	23	25–32	28.83	.42	7.0	......
C. insignis	......	2	4	7	16	8	2	1	......	......	......	......	......	......	......	......	......	......	......	......	......	40	19–25	21.85	.20	5.8	......
Hybrids	......	......	......	2	3	7	8	1	......	......	......	......	......	......	......	......	......	......	......	......	......	21	21–25	23.14	.23	4.6	44
P. clarkii	......	......	......	......	......	5	12	12	7	2	1	......	......	......	......	......	......	......	......	......	......	39	23–28	24.79	.19	4.8	......
Catostomus, species	......	......	......	......	......	......	......	1	1	1	2	......	......	......	......	......	......	......	......	......	......	5	25–28	26.80	.58	4.35	......
Hybrids	......	......	......	......	......	......	......	3	2	10	14	8	7	......	......	......	......	......	......	......	......	44	25–30	27.98	.20	4.85	48
P. santa-anae	......	......	......	......	......	......	......	......	......	6	5	12	17	5	1	......	......	......	......	......	......	46	27–32	29.28	.18	4.25	......
C. tahoensis	......	......	......	......	......	......	......	......	......	......	......	1	4	6	2	1	......	......	......	......	......	14	29–33	30.86	.27	3.3	......
Hybrids	......	......	......	......	......	......	......	......	2	1	3	3	......	......	......	......	......	......	......	......	......	9	26–29	27.78	.40	4.3	60
P. lahontan	......	......	......	......	......	2	5	6	4	8	3	......	......	......	......	......	......	......	......	......	......	28	23–28	25.71	.29	6.0	......
C. catostomus griseus	......	......	......	......	......	......	......	......	......	......	......	1	......	5	5	1	......	......	......	......	......	12	29–33	31.42	.29	3.2	......
Hybrid	......	......	......	......	......	......	......	......	......	......	......	1	......	......	......	......	......	......	......	......	......	1	29	29.0?	......	......	39?
P. jordani	......	......	......	......	......	2	......	2	7	......	......	......	......	......	......	......	......	......	......	......	......	11	23–26	25.27	.36	4.7	......

* Including the counts listed in the top row.

the aberrant specimens are uniformly intermediate between the species which logically may be assumed to be parental. This basis for the identification

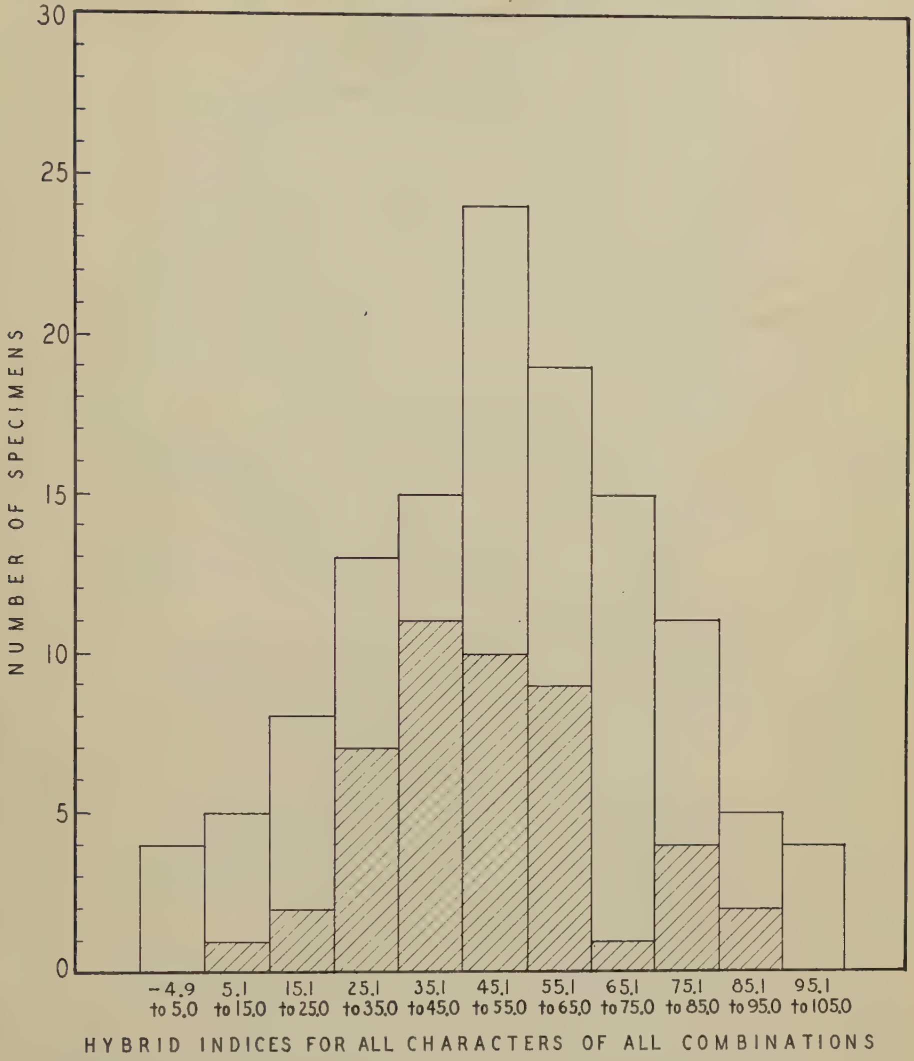

FIG. 8. Histogram showing frequency distribution of the average hybrid index for each hybrid specimen (Table XXXIX).

Shaded rectangles represent *Catostomus* × *Pantosteus* hybrids; the hollow blocks, *Catostomus* × *Catostomus* crosses. The concordant data of 1942 for the *Catostomus*, species × *Pantosteus santa-anae* cross and 18 dorsal ray counts for *Catostomus tahoensis* × *Pantosteus lahontan* were added to the text after the graph was drawn.

of natural hybrids has already been treated (pp. 12–14). We point out now how well the data for the Catostomidae conform with the general observation that, in fishes at least, systematic characters usually exhibit a seemingly almost Galtonian type of inheritance.

Whenever there is a marked and trustworthy difference between the parental species in the number of dorsal rays, the averages for the hybrids intervene nicely between the means for the parental species (Table XXXII). This is also true for the number of scales in each of the 5 series counted (Tables XXXIII–XXXVII; Fig. 7.) In 3 of the combinations the pelvic rays were found to average fewer than in one species but more than in the other (Tables VIII and XXVI).

The proportionate measurements of the hybrids, in general, also intervene between those of the parent forms (Tables II, VI, X, XIV, XVI, XIX, XXII, XXV, XXVIII, and XXX). The evidence is not quite so clear as for the counts, in our opinion principally for the reason that the measurement data need to be separated by localities and size groups, thus greatly restricting the size of each unit of comparison. Even so, the average values are intermediate in the hybrids for almost all the proportions in which the species involved show any considerable difference.

The intermediacy of the hybrids is exhibited also in many features of form and coloration, as outlined through the text and as illustrated in Plates III–VII. As compared with its mate in miscegenation, one of the parental species usually has a larger, more depressed, more bulbous snout, that projects farther beyond the more inferior mouth, and the hybrid is in between. Usually, one of the species is darker and more strongly mottled than the other species, and the hybrid again forms a graded series with its parents. In the *Catostomus commersonnii sucklii* × *Catostomus catostomus griseus* cross, intermediate life colors were also observed (p. 19). In one of the *Catostomus* × *Pantosteus* combinations the hybrids were seen to be intermediate in developmental features of pigmentation (pp. 57–58).

The position of the hybrids between the parental species is strikingly brought out by certain character indices (the use of the character index is explained and justified on pp. 4–6). Indices which sharpen the expression of difference between the species and emphasize the intermediacy of the hybrids are as follows:

Sum of all scale counts minus number of dorsal rays, for the *Catostomus macrocheilus* × *C. syncheilus* combinations (Table V; Fig. 1).

Sum of 3 scale counts minus sum of the other 2 scale counts, for the *Catostomus insignis* × *Pantosteus clarkii* cross (Table XVIII; Fig. 5).

Distance from dorsal fin to occiput divided by length of depressed dorsal fin, for the *macrocheilus* × *syncheilus* series (Table VII; Fig. 2).

Sum of several proportionate measurements of mouth and isthmus (Table XI; Figs. 3–4).

The hybrid indices (pp. 6–8) tend to approximate very closely the value 50, which is the indication of exact intermediacy on a percentage scale, in which the mean value for one parental type is set at 0 and that of the other species at 100. The hybrid indices for the several characters yield a grand average of 51 (Table XXXVIII). The hybrid indices for the charac-

TABLE XXXVIII

FREQUENCIES OF INDIVIDUAL HYBRID INDICES, FOR ALL COMBINATIONS BETWEEN SPECIES OF *Catostomus* AND BETWEEN THOSE OF *Catostomus* AND *Pantosteus*, AND FOR ALL LOCALITIES

An index of 50 indicates exact intermediacy, on a scale in which the average value for the more primitive parental species is set at 0, and that for the more specialized parental species at 100. Indices are disregarded when the difference between the parental species is less than 4 per cent.

Character and Combination	Average Difference between Parent Species*		Individual Hybrid Indices									No.	Ave.†
	Amount	Per Cent	< −37.5	−37.5 to −12.5	−12.5 to 12.5	12.5 to 37.5	37.5 to 62.5	62.5 to 87.5	87.5 to 112.5	112.5 to 137.5	> 137.5		
Dorsal rays													
Catostomus × *Catostomus*	1.8	13	......	......	6	3	36	......	30	......	1	76	63
Catostomus × *Pantosteus*	1.0	5	15	......	......	5	15	1	30	......	9	75	51
Lateral line scales													
Catostomus × *Catostomus*	31.6	45	......	......	......	19	20	7	2	......	......	48	47
Catostomus × *Pantosteus*	8.1	12	4	......	4	8	6	8	5	4	7	46	73
Predorsal scales													
Catostomus × *Catostomus*	20.5	49	......	......	......	5	12	10	2	......	......	29	59
Catostomus × *Pantosteus*	7.2	20	3	6	3	12	23	18	6	2	......	73	43
Scales above lateral line													
Catostomus × *Catostomus*	7.0	55	......	......	......	9	23	8	......	......	......	40	49
Catostomus × *Pantosteus*	2.2	18	19	25	8	13	5	7	7	......	......	84	−8
Scales below lateral line													
Catostomus × *Catostomus*	5.0	52	......	1	1	7	12	16	5	......	......	42	51
Catostomus × *Pantosteus*	1.4	15	6	1	26	2	5	4	3	15	17	79	87
Scales around caudal peduncle													
Catostomus × *Catostomus*	10.6	36	......	......	......	1	24	19	2	......	......	46	59
Catostomus × *Pantosteus*	3.6	15	3	4	12	6	26	14	11	8	......	84	49
Caudal peduncle, depth													
Catostomus × *Catostomus*	3.2	4	3	2	4	7	3	5	4	2	22	52	121
Catostomus × *Pantosteus*	10.2	11	11	4	14	8	18	11	7	3	......	76	26
Head length													
Catostomus × *Catostomus*	16.9	6	6	5	3	8	12	10	10	9	4	67	58
Catostomus × *Pantosteus*	17.2	6	3	......	4	10	12	7	3	3	5	47	63
Head depth													
Catostomus × *Catostomus*	12.3	8	7	4	5	9	19	11	10	5	4	74	50
Catostomus × *Pantosteus*	12.7	8	......	......	1	17	13	6	4	......	......	41	48
Head width													
Catostomus × *Catostomus*	3.6	2	1	2	2	2	......	3	......	1	2	13	47
Catostomus × *Pantosteus*	10.8	7	1	5	5	6	9	21	11	6	5	69	69
Isthmus width													
Catostomus × *Pantosteus*‡	36.7	61	......	......	2	29	33	13	3	......	......	80	45
Snout length													
Catostomus × *Catostomus*	5.4	5	3	1	1	4	6	4	4	2	3	28	57
Catostomus × *Pantosteus*	12.4	11	5	5	4	12	8	19	2	2	11	68	67
Mouth width													
Catostomus × *Catostomus*	7.8	10	14	2	10	4	8	11	2	6	13	70	49
Catostomus × *Pantosteus*	36.2	44	......	2	7	29	24	15	2	1	......	80	43
Cartilaginous ridge, width													
Catostomus × *Catostomus*	7.5	21	3	2	1	14	20	13	8	7	7	75	63
Catostomus × *Pantosteus*	22.9	56	......	......	1	26	44	8	1	......	......	80	44
Mouth length													
Catostomus × *Catostomus*	3.3	6	14	1	9	1	2	9	6	8	8	58	48
Catostomus × *Pantosteus*	24.7	42	3	1	......	18	28	13	12	1	3	79	57
Lower lip, union													
Catostomus × *Catostomus*	3.6	30	13	13	4	13	7	13	5	5	3	76	26
Catostomus × *Pantosteus*	22.1	141	......	......	4	31	37	7	1	......	......	80	30
Dorsal fin to occiput, distance§													
Catostomus × *Catostomus*	19.6	8	4	5	6	6	20	13	7	6	4	71	52
Dorsal fin base§													
Catostomus × *Catostomus*	32.0	14	1	4	6	10	20	23	11	1	......	76	52
Depressed dorsal fin, length§													
Catostomus × *Catostomus*	29.3	10	4	2	6	14	21	22	3	1	1	74	44
All counts													
Catostomus × *Catostomus*	....	39	......	1	7	44	127	60	41	......	1	281	55
Catostomus × *Pantosteus*	....	14	50	36	53	46	80	52	62	29	33	441	47
All measurements													
Catostomus × *Catostomus*	....	10	73	43	57	92	138	137	70	53	71	734	54
Catostomus × *Pantosteus*	....	39	23	17	42	186	226	120	46	16	24	700	48
All counts and measurements	....	2–30	143	93	144	194	314	253	189	96	126	1552	53
	....	36–141	3	4	15	174	257	116	30	2	3	604	47
Grand summary	....	23	146	97	159	368	571	369	219	98	129	2156	51

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* The averages in these two columns are weighted by number of specimens, and differences of less than 4 per cent are considered in these computations. The amount of difference refers to the actual counts, or to measurements expressed in thousandths of the standard length.

† Weighted averages, based on the original, unclassed data.

‡ This measurement differs little, and was not taken for the interspecific hybrids, within the genus *Catostomus*.

§ These measurements were taken only for the *Catostomus* × *Catostomus* hybrids.

TABLE XXXIX

FREQUENCIES OF AVERAGE HYBRID INDEX FOR EACH INDIVIDUAL OF EACH COMBINATION

An average index of 50 indicates exact intermediacy, on a scale in which the average value for the more primitive parental species is set at 0, and that for the more specialized parental species at 100. Indices are disregarded when the difference between the parental species is less than 4 per cent.

Hybrid Combination	Average Hybrid Index for Each Individual											No.	Ave.*
	−4.9 to 5.0	5.1 to 15.0	15.1 to 25.0	25.1 to 35.0	35.1 to 45.0	45.1 to 55.0	55.1 to 65.0	65.1 to 75.0	75.1 to 85.0	85.1 to 95.0	95.1 to 105.0		
Catostomus commersonnii sucklii × *Catostomus catostomus griseus*	......	1	......	1	......	......	2	......	1	......	......	5	49
Catostomus macrocheilus × *Catostomus syncheilus syncheilus*	4	3	6	5	4	13	6	14	6	3	4	68	53
Catostomus macrocheilus × *Catostomus syncheilus palouseanus*	......	......	......	......	......	1	2	......	......	......	......	3	56
Catostomus commersonnii sucklii × *Pantosteus jordani*	......	......	......	......	1	1	......	......	......	......	......	2	43
Catostomus commersonnii sucklii × *Pantosteus delphinus delphinus*	......	......	......	2	1	1	1	......	2	......	......	7	53
Catostomus insignis × *Pantosteus clarkii*	......	......	......	3	6	7	4	1	......	......	......	21	48
Catostomus, species × *Pantosteus santa-anae*	1	3	5	5	6	9	5	2	1	1	......	38	41
Catostomus tahoensis × *Pantosteus lahontan*	......	1	2	2	1	1	1	......	1	1	......	10	44
Catostomus catostomus griseus × *Pantosteus jordani*	......	......	......	......	......	1	......	......	......	......	......	1	45
Catostomus latipinnis discobolus × *Pantosteus delphinus utahensis*	......	......	......	......	1	......	......	......	......	......	......	1	43
Totals													
Catostomus × *Catostomus*	4	4	6	6	4	14	10	14	7	3	4	76	53
Catostomus × *Pantosteus*	1	4	7	12	16	20	11	3	4	2	......	80	45
Grand totals	5	8	13	18	20	34	21	17	11	5	4	156	49

* Based on original, unclassed data.

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ter indices of mouth and lip structures average 42 (Table XII). The averages of the summary hybrid indices for all the characters in each individual vary from 41 to 56 for each of the 10 combinations, with a grand average of 49—again very close to exact intermediacy (Table XXXIX; Fig. 8).

For a few characters the hybrid indices tend toward a trimodal distribution, with modes below 0 and near or above 100 as well as the submeridian one (Table XXXVIII). This is true of the indices for dorsal ray counts, despite the fact that the average number of rays of this fin in the hybrids lies between the means for the parental species (Table XXXII). The reason is that the averages for the parental species of certain crosses differ by less than 1.0 ray so that the counts for the hybrid, necessarily whole numbers, often fall beyond the mean for either parental species. The hybrid counts must all fall beyond either the one or the other of the parental means in the combination, *Catostomus tahoensis* × *Pantosteus lahontan,* for in this cross the means for the parental species, respectively 10.38 and 10.10, both lie between two integers; if the hybrid count is 10 the index will be above 100, if 11, below 0. A tendency toward extreme indices is also shown for the counts of scales above and below the lateral line and for the depth of the caudal peduncle, because the *Catostomus,* species × *Pantosteus santa-anae* hybrids tend to have high values for these characters (see p. 49). The hybrid indices cluster close about the mean value of 50, for the characters by which the parental species on the average differ by more than 30 per cent (Table XXXVIII, next to last row).

The tendency toward intermediacy pervades the whole structural system of the organism, involving internal as well as external characters. In this respect, too, the sucker hybrids agree with interspecific crosses in other fish families—for instance in the Pleuronectidae (Hubbs and Kuronuma, 1942). The intercalation of the catostomid hybrids between the parental species in structural features is particularly well shown by the mouth characters (as demonstrated through the preceding text, in Tables XI–XII, in Figs. 3–4; and as illustrated in Pls. I, II, and IV). Differences in color of peritoneum, length of intestine, and size of air bladder are also involved. As shown by Figure 6 and Table XXIII the degree of development of the parietal fontanelle is intermediate in the hybrid between *Catostomus,* species and *Pantosteus santa-anae* (the 2 larger *C. commersonnii* × *P. delphinus* hybrids fail to show such intermediacy, and the *C. insignis* × *P. clarkii* cross was not studied in this regard). No other explanation than that of hybridization would seem capable of explaining the interposition of the whole array of characters between those of 2 species.

An intermediate type of relative growth in 1 hybrid is indicated by plotting the character index based on the lip and isthmus structures (Fig. 4).

In all ways, therefore, the hybrids tend to be intermediate between the species which miscegenated to produce them, and the intermediacy is often highly specific; that is, the hybrids have characters which lie between those

TABLE XL

Frequencies of Coefficients of Variability (V) in Catostomid Hybrids and in the Parental Species

	Coefficients of Variability								No.	Ave.*
	2.0 to 3.9	4.0 to 5.9	6.0 to 7.9	8.0 to 9.9	10.0 to 11.9	12.0 to 13.9	14.0 to 15.9	16.0 to 17.9		
Ray counts (Tables VIII, XXVI, XXXII)										
Parental species 1†	4	9	......	......	......	......	......	......	13	4.5
Hybrids	2	8	......	......	......	......	......	......	10	4.6
Parental species 2‡	3	10	......	......	......	......	......	......	13	4.5
Scale counts (Tables XXXIII–XXXVII)										
Parental species 1	2	12	22	7	2	......	......	......	45	6.7
Hybrids	1	9	8	4	2	......	3	4	31	8.8
Parental species 2	......	11	15	13	4	1	......	1	45	7.8
Character index, scale and ray counts (Table V)										
Parental species 1	......	1	1	......	......	......	......	......	2	6.1
Hybrids	......	1	......	......	......	......	......	......	1	4.6
Parental species 2	......	2	......	......	......	......	......	......	2	4.8
Character index, scale counts (Table XVIII)										
Parental species 1	......	......	......	1	......	......	......	......	1	8.0
Hybrids	......	1	......	......	......	......	......	......	1	5.1
Parental species 2	......	1	......	......	......	......	......	......	1	5.7
Character index, proportions (Tables VII and XI)										
Parental species 1	2	4	3	......	......	......	......	......	9	5.4
Hybrids	......	2	1	3	......	......	......	......	6	6.9
Parental species 2	1	2	3	......	2	......	1	......	9	8.0
Totals, for counts and indices										
Parental species 1	8	26	26	8	2	......	......	......	70	6.1
Hybrids	3	21	9	7	2	......	3	4	49	7.5
Parental species 2	4	26	18	13	6	1	1	1	70	7.1

* Based on original, unclassed data.

† The coarse-scaled *Catostomus* in the *Catostomus* × *Catostomus* crosses and the *Catostomus* species in the *Catostomus* × *Pantosteus* hybrids.

‡ The fine-scaled *Catostomus* in the *Catostomus* × *Catostomus* hybrids and the *Pantosteus* species in the *Catostomus* × *Pantosteus* crosses.

of the particular forms with which they are associated. This particularized intermediacy may be seen in external features of general appearance (as shown on Pls. III to VII); particularly well in mouth structures (p. 34; Pls. I–II); also in size of scales, including the squamational pattern (pp. 22, 30, 37, 40, and 43; Pl. V; Fig. 7), and in many other characters. By such relations the interpretation of the variant suckers as hybrids is strongly verified.

As evidence of hybridity, a point of outstanding importance is that several uncorrelated characters in the same set of specimens show intermediacy between the same pair of species. Thus, the suckers of the Columbia River system which are intermediate between *C. macrocheilus* and *C. syncheilus* in the number of scales and dorsal fin rays (Fig. 1) also show intervening values in the relative length of the depressed dorsal fin and of the distance forward to the occiput (Fig. 2). The uniform tendency toward intermediacy is witnessed by almost the entire body of data in the present study.

All of the data on the interspecific hybrids in the Catostomidae therefore conform to the rule that simple Mendelian segregation is seldom displayed in the inheritance of the systematic characters of fishes (Hubbs, 1940: 205–9). This rule holds whether the differences between the parental species be qualitative or quantitative and whether the differences be large and striking, or so minor as to be demonstrable only by statistical analysis. Uniformly, the offspring exhibit intermediate characteristics. They do not grade from the one extreme of one parental species to the opposite extreme of the other form involved in the cross: for any character the frequency distribution is in general similar to that displayed by each parental species (Figs. 1–3, 5, 7; Tables V, VII, VIII, XI, XVIII, XXVI, XXXII–XXXVII). The tendency for the hybrid indices to cluster about the point (50) of exact intermediacy (Tables XII, XXXVIII–XXXIX; Fig. 8) confirms the same principle. There is, of course, a theoretical possibility that Mendelian segregation may obtain, for there is a conceivable (though remote) chance that all characters show a balance between dominance and recessiveness.

For each character and for all characters combined, the hybrids seem to show little if any increase in variability as compared with the parental species. This conclusion, based on an inspection of the figures and tables, has been confirmed by computing and summarizing the coefficients of variability (V). The coefficients have the same order of range and of magnitude in the hybrids that they do in the parental species (Table XL). The slightly higher average coefficients shown by the hybrids for certain features is to be explained as a consequence of the smaller samples, which cause the values of V to fluctuate; a few values are sufficiently high to raise the average a little.

The fact that the coefficients of variability are not notably increased in the hybrids conforms to the principle that systematic characters show a seemingly Galtonian type of inheritance. Obviously there has been no large amount of error in the identification of the hybrids, for if there had been many mistakes, the variability would have been greater. Clearly, too, there can have been little backcrossing between the hybrids and the parental species, for this would also have led to a marked increase in variability.

Since, so far as we have discovered, there has been little if any backcrossing between the catostomid hybrids and their parental species and no real hybrid populations have been built up, we are unable to indicate that interspecific hybridization has played a role of any speciational importance in the Catostomidae. The fact that *Catostomus syncheilus* is intermediate in many respects between *Catostomus macrocheilus* and *Pantosteus jordani*—two other species of the Columbia River system—suggests the possibility that it may have arisen through the hybridization of those forms. *C. syncheilus,* however, shows its full specific distinctness in certain peculiar features, by its occurrence in many places with only one or with neither of the other species, and by the fact that it breaks up into its own series of local races. The approach toward *Pantosteus* also displayed by *Catostomus catostomus* conceivably could have been due to past hybridization, followed by backcrossing and inbreeding. It is our opinion, however, that the characters which *Catostomus syncheilus* and *C. catostomus* share with *Pantosteus* are the products of parallel evolution, for all of these features can be related to life on the bottom of swift, shallow streams. Such parallelisms are abundantly illustrated by fresh-water fishes (Hubbs, 1940, 1941*b*).

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PLATE I

Mouths of sucker hybrids and parental species

Drawn by Grace Eager

The enlargements were adjusted so that the specimens of the parental species (*a* and *c*) were represented as if they were of the same size as the hybrid (*b*).

FIG. 1*a*. *Catostomus*, species: U.M.M.Z. No. 131665, 153 mm. in standard length, from Sespe Creek, Ventura County, California.

FIG. 1*b*. Hybrid: U.M.M.Z. No. 131666, 114 mm. long, from the same collection.

FIG. 1*c*. *Pantosteus santa-anae:* U.M.M.Z. No. 131657, 95 mm. long, from pond off Piru Creek, Ventura County, California.

FIG. 2*a*. *Catostomus tahoensis:* U.M.M.Z. No. 124855, 83 mm. long, from Carson River, Churchill County, Nevada.

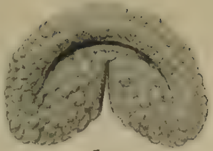
FIG. 2*b*. Hybrid: U.M.M.Z. No. 124861, 84 mm. long, from the same collection.

FIG. 2*c*. *Pantosteus lahontan:* U.M.M.Z. No. 124856, 84 mm. long, from the same collection.

FIG. 3*a*. *Catostomus insignis:* U.M.M.Z. No. 121641, 91.5 mm. long, from Middle Fork of East Branch of Black River, Apache National Forest, Arizona.

FIG. 3*b*. Hybrid: U.M.M.Z. No. 121642, 94 mm. long, from the same collection.

FIG. 3*c*. *Pantosteus clarkii:* U.M.M.Z. No. 121640, 90.5 mm. long, from the same collection.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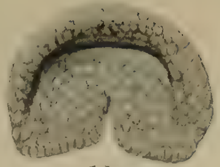
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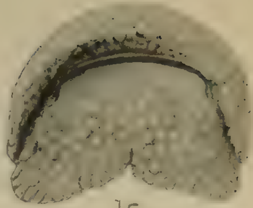
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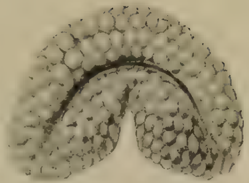
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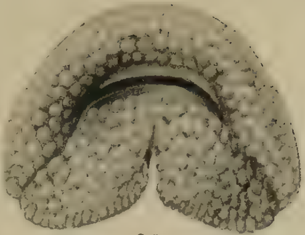
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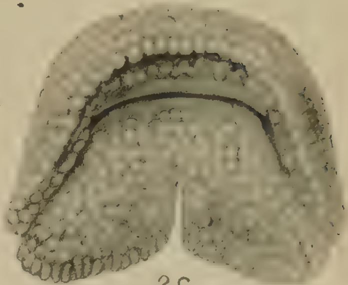
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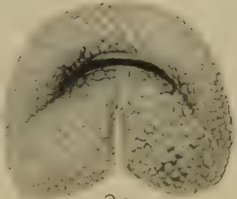
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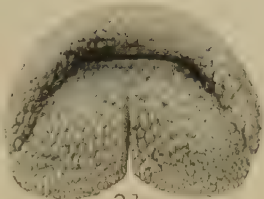
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G. Eager

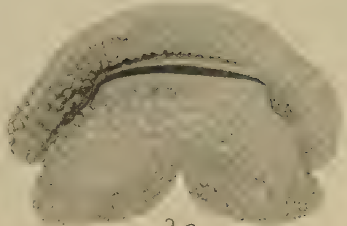
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PLATE III

Hybrid sucker, *Catostomus commersonnii sucklii* × *Catostomus catostomus griseus,* and the parental species

Photographed by Clarence Flaten

FIG. 1. *Catostomus commersonnii sucklii:* U.M.M.Z. No. 132229, 155 mm. in standard length, from Laramie River, Albany County, Wyoming.

FIG. 2. Hybrid: U.M.M.Z. No. 132230, 152 mm. long, from the same collection.

FIG. 3. *Catostomus catostomus griseus:* U.M.M.Z. No. 132217, 156 mm. long, from North Platte River, east of Parco, Carbon County, Wyoming.

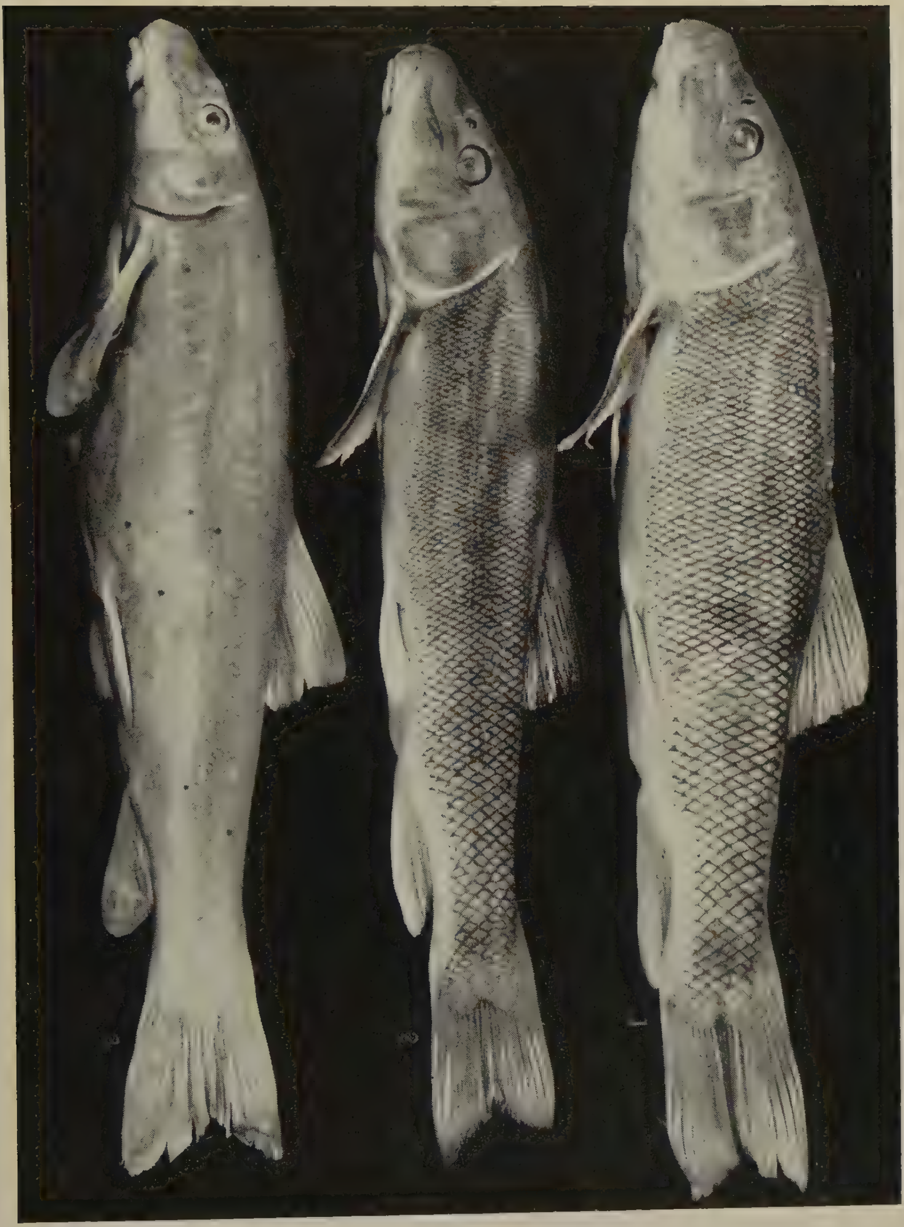


PLATE IV

Hybrid sucker, *Catostomus commersonnii sucklii* × *Pantosteus jordani,* and the parental species

Photographed by Clarence Flaten

Fig. 1. *Catostomus commersonnii sucklii:* U.M.M.Z. No. 132236, 55 mm. in standard length, from Lodgepole Creek, Kimball County, Nebraska.

Fig. 2. Hybrid: U.M.M.Z. No. 120375, 56 mm. long, from Grace Coolidge Creek, Custer County, South Dakota.

Fig. 3. *Pantosteus jordani:* U.M.M.Z. No. 120378, 55 mm. long, from Stockade Beaver Creek, Weston County, Wyoming.

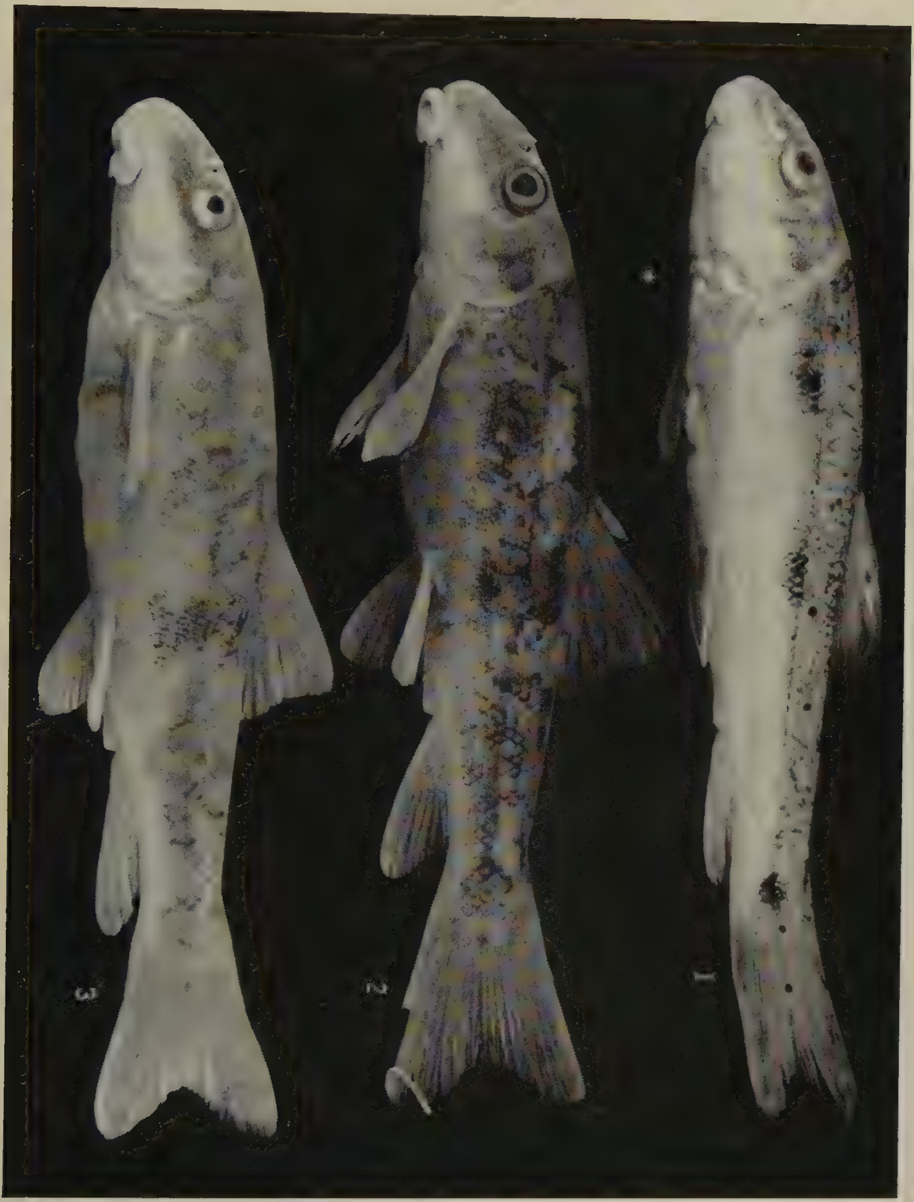

3
2
1

PLATE V

Hybrid sucker, *Catostomus commersonnii suckliii* × *Pantosteus delphinus delphinus,* and the parental species

Photographed by Clarence Flaten

FIG. 1. *Catostomus commersonnii sucklii:* U.M.M.Z. No. 132218, 127 mm. in standard length, from the North Platte River, Carbon County, Wyoming.

FIG. 2. Hybrid: U.M.M.Z. No. 136912, 128 mm. long, from Dry Creek, 3 miles west of Olathe, Montrose County, Colorado.

FIG. 3. *Pantosteus delphinus delphinus:* U.M.M.Z. No. 113424, 123 mm. long, from Green River, at Green River, Wyoming.

PLATE V

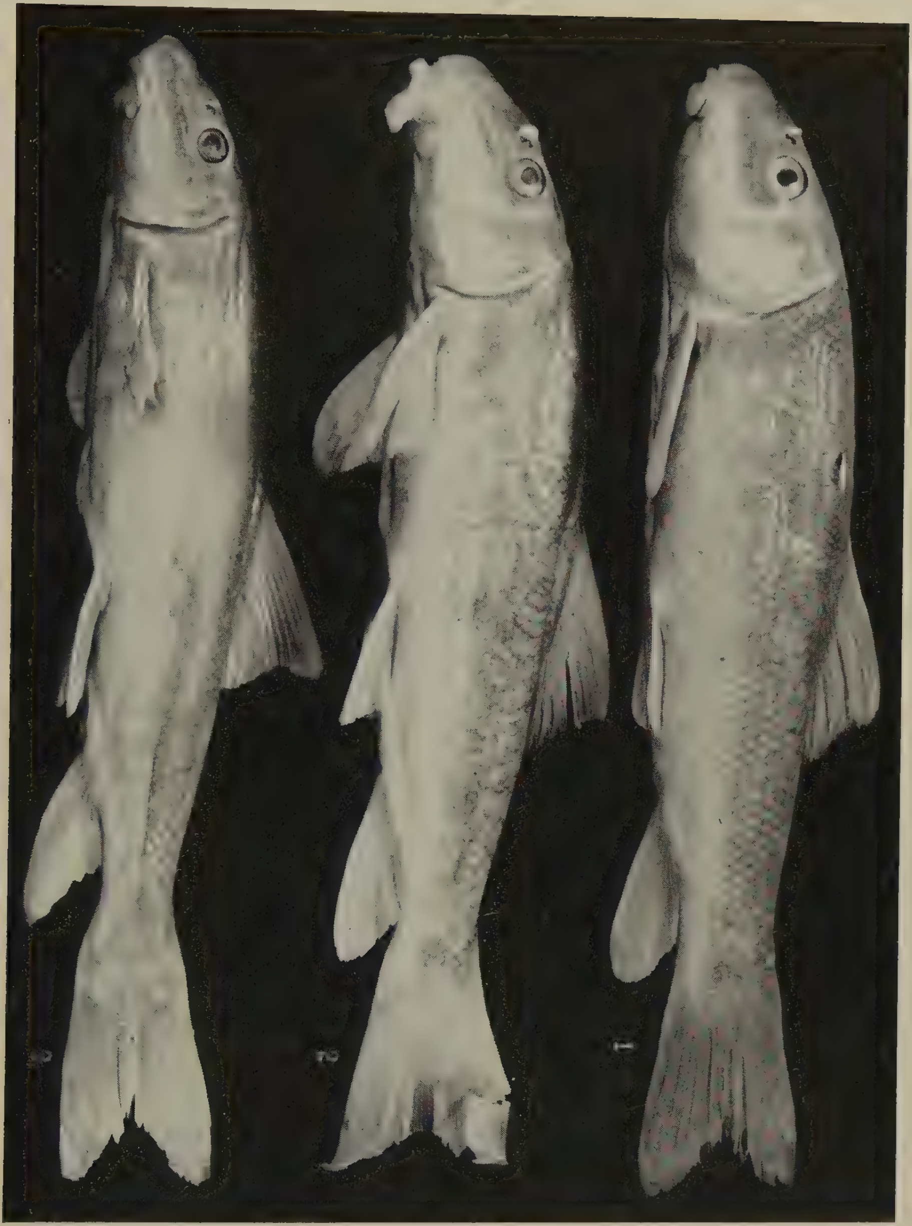


PLATE VI

Heads of sucker hybrids and their parental species

Photographed by Clarence Flaten

Fig. 1*a*. *Catostomus macrocheilus:* U.M.M.Z. No. 130470, 149 mm. in standard length, from Sucker Creek above Rockville, Malheur County, Oregon.

Fig. 1*b*. Hybrid: U.M.M.Z. No. 130471, 150 mm. long, from the same collection.

Fig. 1*c*. *Catostomus syncheilus syncheilus:* U.M.M.Z. No. 130469, 138 mm. long, from the same collection.

Fig. 2*a*. *Catostomus macrocheilus:* U.M.M.Z. No. 98721, 202 mm. long, from Smokle Creek, north of Almota, Whitman County, Washington.

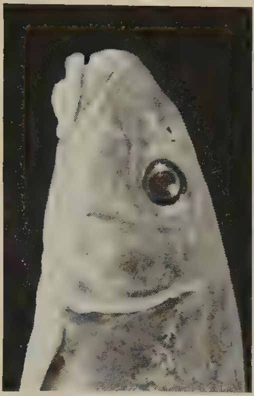
Fig. 2*b*. Hybrid: U.M.M.Z. No. 98709, 204 mm. long, from South Fork of the Palouse River, Whitman County, Washington.

Fig. 2*c*. *Catostomus syncheilus palouseanus:* U.M.M.Z. No. 98707, 178 mm. long, from the same collection.

Fig. 3*a*. *Catostomus insignis:* U.M.M.Z. No. 110431, 105 mm. long, from Taylor Creek, Gila National Forest, New Mexico.

Fig. 3*b*. Hybrid: U.M.M.Z. No. 110433, 110 mm. long, from the same collection.

Fig. 3*c*. *Pantosteus clarkii:* U.M.M.Z. No. 124743, 111 mm. long, from Gila River at Cliff, Grant County, New Mexico.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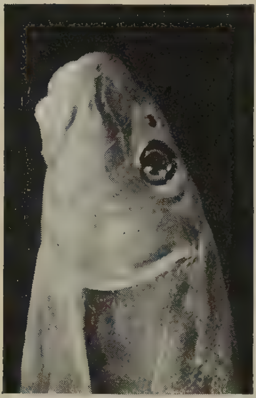
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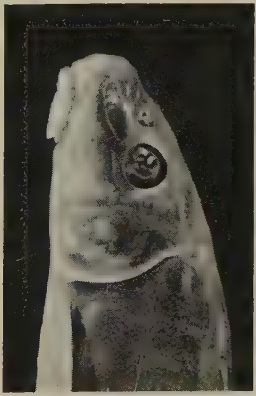
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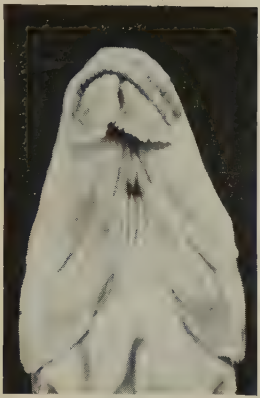
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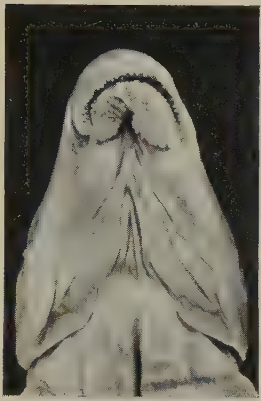
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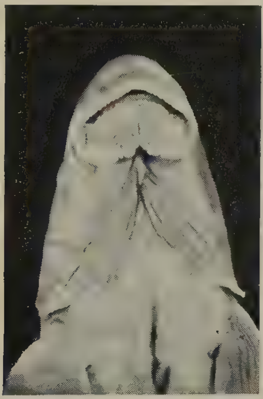
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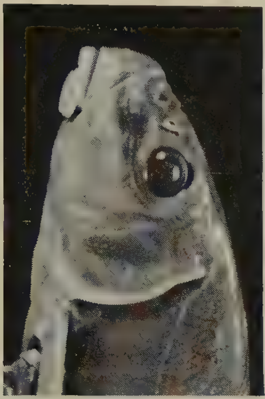
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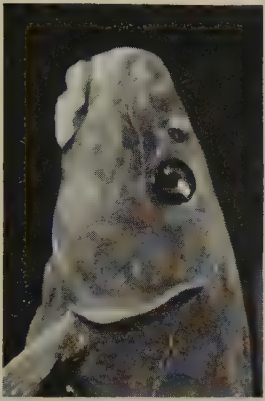
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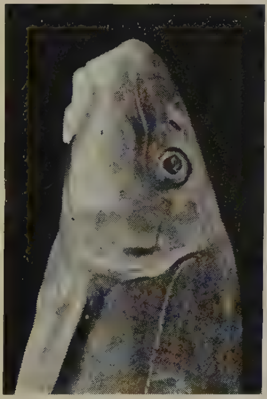
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PLATE VII

Heads of sucker hybrids and their parental species

Photographed by Clarence Flaten

FIG. 1*a*. *Catostomus*, species: U.M.M.Z. No. 133857, 119 mm. in standard length, from Santa Clara River, Ventura County, California.

FIG. 1*b*. Hybrid: U.M.M.Z. No. 131666, 114 mm. long, from Sespe Creek, Ventura County, California.

FIG. 1*c*. *Pantosteus santa-anae:* U.M.M.Z. No. 131756, 115 mm. long, from North Branch of Warm Spring Creek, San Bernardino County, California.

FIG. 2*a*. *Catostomus tahoensis:* U.M.M.Z. No. 124912, 114 mm. long, from Humboldt River, Elko County, Nevada.

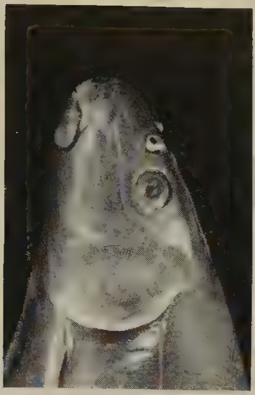
FIG. 2*b*. Hybrid: U.M.M.Z. No. 133688, 122 mm. long, from Little Truckee River, Nevada County, California.

FIG. 2*c*. *Pantosteus lahontan:* U.M.M.Z. No. 124869, 119 mm. long, from Humboldt River at Irish-American Dam, Pershing County, Nevada.

FIG. 3*a*. *Catostomus catostomus griseus:* U.M.M.Z. No. 132217, 95 mm. long, from North Platte River, Carbon County, Wyoming.

FIG. 3*b*. Hybrid: U.M.M.Z. No. 127541, 98 mm. long, from Sweetwater River, Fremont County, Wyoming.

FIG. 3*c*. *Pantosteus jordani:* U.M.M.Z. No. 127540, 99 mm. long, from the same collection.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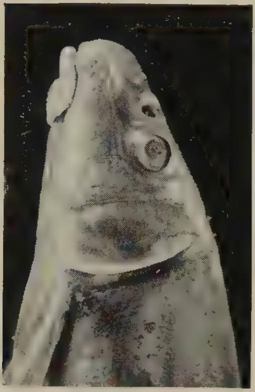
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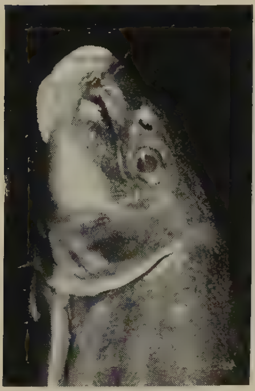
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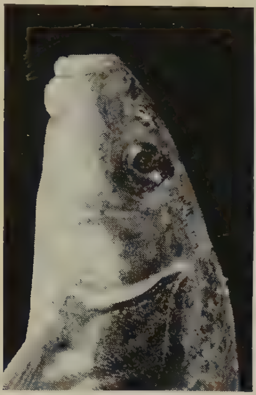
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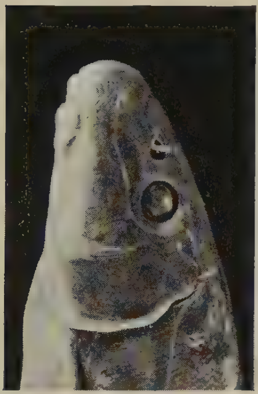
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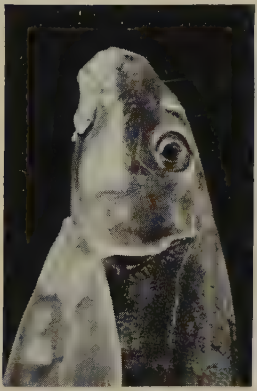
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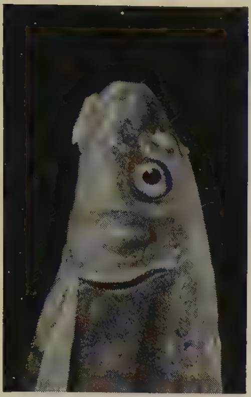
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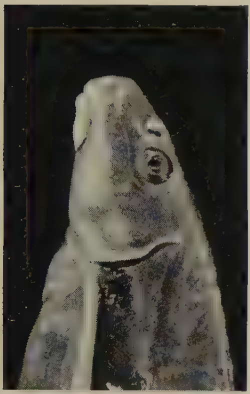
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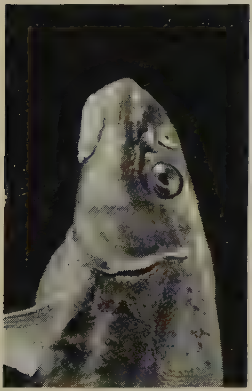
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CONTRIBUTIONS FROM THE LABORATORY OF VERTEBRATE BIOLOGY

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HYBRIDIZATION IN NATURE BETWEEN SPECIES OF AMERICAN CYPRINODONT FISHES

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INTRODUCTION

THIS study is one of a series, in which an attempt is being made to analyze the hybridization in nature between species of fish. The aims and methods presented in the recent papers by Hubbs and Kuronuma (1942) and by Hubbs, Hubbs, and Johnson (1943) have been followed. Pertinent literature references are included in those papers.

Two of the three hybrid combinations described on the following pages are treated as intergeneric. In all three sets the parental forms are undoubtedly very well-marked species, but whether any or all should be treated as generically distinct is a matter of taxonomic judgment. Until recently, in fact, both members of each parental pair now regarded as generically distinct were customarily referred to a single genus. Thus *Chriopeops goodei* and *Lucania parva* were both classed in *Lucania,* and *Plancterus kansae* as well as *Fundulus sciadicus* were both put in *Fundulus,* by Jordan and Evermann (1896: 632–66; and 1898: 2828) and by other authors. The discovery of new characters, along with a different interpretation of long-known features, has led recent revisers of the Cyprinodontidae to treat *Chriopeops* as generically distinct from *Lucania,* and *Plancterus* as separable from *Fundulus* (Myers, 1924: 8; 1925: 370; and 1931: 10; Hubbs, 1926: 5, 14).

Until the specimens here discussed were discovered, we knew of no natural hybrids in the oviparous cyprinodonts. We had already become convinced that Weed (1921: 70) was in error in reporting an extensive local hybridization between three species of *Fundulus.* His remarks were as follows:

> . . . Under certain conditions hybridization in nature is very common.
>
> The writer once found in some pools near Chesapeake Beach, Maryland, very large numbers of three species of *Fundulus* breeding in very restricted waters. Hundreds, if not thousands of fish were emitting spawn and milt at the same time in a space of not much over a hundred square feet of water a few inches deep. About half of the fish taken in those pools showed such intergrading characters that they could not be identified.

It seemed inherently improbable that three species would engage in such promiscuous spawning, and no other natural hybrids in *Fundulus* were known. The senior writer therefore undertook to check the case. Mr. Weed kindly furnished the information that representative specimens had been left at the United States National Museum. A search there, among uncatalogued as well as catalogued specimens of the three species of *Fundulus* involved (*F. diaphanus, F. majalis,* and *F. heteroclitus*), disclosed much material collected by Mr. Weed near Chesapeake Beach, but failed to uncover a single apparent hybrid. Field collecting, in localities said by Earl D. Reid to be the places where Mr. Weed seined, yielded an abundance of the three forms just named. After due allowance was made for age and sex differences, every fish could be identified, as one of the three species rather than as a hybrid. We now report, however, one hybrid between *F. diaphanus* and *F. heteroclitus.*

Incidentally, the similar mass hybridization between "*Notropis heterodon, Notropis cayuga, Notropis anogenus,* etc.," indicated by Weed (1921: 69–70) as probably occurring in Turtle Creek, Wisconsin, and in Lake Ontario, failed of confirmation when the material was re-examined. The difficulty with the Wisconsin collection lay largely in the inclusion of a form, *Notropis xaenocephalus richardsoni* (Hubbs and Greene), which was unknown in 1921.

Natural hybrids, however, are known to occur in the Poeciliidae, a family of viviparous cyprinodonts (Hubbs and Hubbs, 1932; Hubbs, 1933; and 1936: 245–46, Pl. 8, Fig. 4). Hybridization in this family is being subjected to an extensive experimental study, with results that have recently been summarized (Hubbs, 1940: 205–9).

The single hybrid between *Fundulus diaphanus diaphanus* and *Fundulus heteroclitus macrolepidotus* was found in a large collection from Prince Edward Island, sent to us by M. W. Smith of the Fisheries Research Board of Canada. It came to light after this paper had been written. The *Chriopeops* × *Lucania* hybrids were discovered by Boyd W. Walker, who made the counts and measurements for this combination. The specimens were collected in Florida by Carl L. Hubbs, Laura C. Hubbs, and Boyd W. Walker. The *Fundulus* × *Plancterus* cross was found by Raymond E. Johnson in the course of his survey of the fish fauna of Nebraska, and he made the comparison between the single known hybrid specimen and the parental species.

All specimens used in this study are deposited in the fish collection of the University of Michigan Museum of Zoology.

The methods of counting and measuring follow those outlined by Hubbs and Lagler (1941: 12–20, Figs. 2–3). As in two previous papers, by Hubbs and Kuronuma (1942: 291–94) and by Hubbs, Hubbs, and Johnson (1943: 6–8), we find it very useful to compute a hybrid index, for each character of each specimen. This index denotes the position of the hybrid between the parental species, on a percentage scale in which the average value for the more primitive parental kind is set at 0 and that for the other form at 100. In the present study *Fundulus diaphanus* is interpreted as less specialized than *Fundulus heteroclitus; Chriopeops* as more primitive than *Lucania; Plancterus* as a derivative of *Fundulus.* The several hybrid indices of all specimens are averaged for each character, and all the character indices for each fish are averaged for each individual. A grand average is then obtained, to indicate the relations of all the hybrids to the parental species in all characters that were counted and measured.

Some characters that were counted and measured have not been tabulated. The data were discarded when no significant difference between the parental species was indicated, or when the ranges of variation for the two parental species overlapped very widely and the difference between the hybrids and either parental type was much less than the range of variation for either parent.

Fundulus diaphanus diaphanus × *Fundulus heteroclitus macrolepidotus*

(Pls. I–III)

U.M.M.Z. No. 138833: 1 adult female discovered in a large collection of *Fundulus* seined by M. W. Smith of the Fisheries Research Board of Canada on the shoals of Lake of Shining Waters, on Prince Edward Island, in the Gulf of St. Lawrence, on August 23, 1939.

Dr. Smith informs us that this is a fresh-water lake with a pH of 8.9 at the time and place of collecting; water temperatures ran from 17.5° to 26.6° C.; emergent and submerged vegetation grew plentifully, and the shores were also vegetated.

Concerning the ecology of Lake of Shining Waters and other "ponds" on Prince Edward Island in which large samples of *Fundulus* were taken in a fishery biology survey, Dr. Smith supplies further information, as follows. All the lakes lie just behind the mouths of small streams, back of barriers built up above high tide level. Salt water does not spill into these ponds at the normal high tide, but in times of storm varying amounts of sea water may wash in. Records for Clark Pond indicate a variation in salinity from 0.7 to 22.2 parts per mille. Dalvay, Long, and Shining Waters are virtually fresh-water habitats, and have been found to contain only traces of salt water, although it is known that the latter 2 ponds have at times been flooded. The areas and maximum depths are: Lake of Shining Waters, 8.1 hectares, 2.2 meters; Clark Pond, 12.1 hectares, 3.0 meters; Long Pond, 19.8 hectares, 2.0 meters; Dalvay Pond, 22.1 hectares, 3.7 meters. Aquatic vegetation is plentiful in all the ponds—*Sparganium* and *Typha* along the shores, and *Potamogeton* and *Myriophyllum* in extensive beds within the water. In the ponds other than Lake of Shining Waters the temperatures at the time of collecting ranged from 22.2° to 25.5° C., and the pH from 8.4 to 9.0. All series were taken August 23, 1939. All of the collections were made with a seine and were entirely random in regard to the species of *Fundulus*. In fact, it was not recognized in the field that 2 species (and the 1 hybrid) were included. It is clear, therefore, that *Fundulus diaphanus diaphanus* was the dominant form of the genus, and very abundant, whereas the essentially salt- and brackish-water *Fundulus heteroclitus macrolepidotus* was very scarce (Table I).

The lakes of Prince Edward Island are obviously *diaphanus* habitats, into which a small number of the bay-shore form *heteroclitus* has wandered.

TABLE I

RELATIVE NUMBERS OF HYBRIDS, *Fundulus diaphanus diaphanus* × *Fundulus heteroclitus macrolepidotus*, AND OF THE PARENTAL SPECIES, IN COLLECTIONS FROM THE LAKES OF PRINCE EDWARD ISLAND

Lake	*Fundulus diaphanus diaphanus*	Hybrids	*Fundulus heteroclitus macrolepidotus*
Lake of Shining Waters	637 (97.5%)	1 (0.2%)	15 (2.3%)
Clark Pond	262	......	36
Long Pond	2295	......	9
Dalvay Pond	616	......	......
In all 4 collections	3810 (98.4%)	1 (0.03%)	60 (1.6%)

The unusually robust form of the males in these collections of *Fundulus diaphanus diaphanus*[1] suggested to Dr. Smith that there might have been some hybridization in the Prince Edward Island lakes between *F. diaphanus* and *F. heteroclitus*. We were unable to verify the suspicion that this character was due to hybridization, but on carefully examining the 4 large series we did locate the 1 hybrid. Its status can hardly be open to question, for it is thoroughly different from and definitely intermediate between the 2 assumed parental species. No other cyprinodont ranges into the Maritime Provinces.

This single hybrid specimen is intermediate in color tone, coloration, general body form, and proportions; in the gillraker, scale, and fin-ray numbers; and in certain superficial structures. The characters subjected to statistical treatment bring out precisely the intermediacy of the hybrid (Table II). The grand average hybrid index for the characters analyzed in Table II is 50, indicating that in these respects the hybrid is on the average exactly intermediate between the parental species.

---

[1] The subspecific characters of *Fundulus diaphanus diaphanus* have been stated by Hubbs and Lagler (1941: 67).

In all the proportionate measurements the values for the hybrid lie between those for the parental types. There is no overlap for 11 items (body and caudal peduncle depths, predorsal and prepelvic lengths, height of anal sheath, head depth and width, interorbital and mouth widths, diameter of nonimbricated scale, and 1 of the pore measurements). The position of the dorsal fin (Pl. I) is a particularly obvious character of the hybrid: when measured forward the distance from the caudal base to the origin of the dorsal fin extends to the posterior part of the eye in *F. d. diaphanus* (to the posterior margin of the orbit in 1), to just behind the eye in the hybrid, and to about the middle of the opercle in *F. heteroclitus macrolepidotus.* The hybrid value is also intermediate, but shows a slight overlap on the measurements of one or both parental species, in 6 characters (length of depressed dorsal fin, of snout, and of orbit, upper lip width, and 2 of the pore measurements). The character index based on the 3 pore measurements (Pl. III), however, intervenes, with a wide gap on either side. The pattern formed by the canals and pores on the top of the head is one of the clearly intermediate characters. The ratio of the head depth to orbit length in the hybrid is distinctive, without overlap. The angle between the head contours barely overlaps the values for *F. d. diaphanus.* The displacement index, measuring the obviously different relative bulks of the bodies (all eviscerated), is interpolated between the ranges for the parental species, without overlap, though *diaphanus* is approached more closely than is *heteroclitus* in this respect.

The intermediacy of the hybrid is confirmed by the gillraker, scale, and ray counts. The raker counts are 5 to 7, usually 6 for *diaphanus,* 8 for the hybrid, and 9 to 12 for *heteroclitus.* The number of transverse rows from the upper end of the gill slit to the base of the caudal fin is particularly distinctive in the hybrid; 41 as contrasted with 34 to 36 for the local stocks of *heteroclitus* and 44 to 51 for the Prince Edward Island *diaphanus.* The caudal ray count (branched rays plus 2) is also consistently intermediate in the hybrid. Some overlap is shown by the other scale and ray counts.

The hybrid origin of the specimen under discussion is further confirmed by a peculiar pigmentary character. Over the entire exposed surface of the scales on the top of the head there are developed, in the *F. d. diaphanus* specimens, numerous minute, seemingly melanotic tubercles. These come into clear view when the surface of the head is quickly desiccated, as by a jet of compressed air. Under this treatment the ordinary melanophores, lying deeper in the skin, become scarcely apparent (Plate III shows the melanophores and hence does not bring out the tiny, black, superficial swellings). In the examples of *F. heteroclitus macrolepidotus* these melanotic (?) structures are barely evident, and are confined to the periphery of the scale. In the hybrid they are rather well developed, but are less prominent than in the *diaphanus* material.

TABLE II

Comparison of the *Fundulus* Hybrid with the Parental Species

Based on 15 adult females from Lake of Shining Waters, Prince Edward Island: 10 of *F. diaphanus diaphanus* (9 for the nonimbricated scale measurement), the 1 hybrid, and the 4 comparable females of *F. heteroclitus macrolepidotus* from this lake (11 adults used for the gillraker count).

	Fundulus diaphanus diaphanus	Hybrid		*Fundulus heteroclitus macrolepidotus*
	Range (Average)	Proportions and Counts	Hybrid Index	Range (Average)
Standard length, mm.	51.0–55.5 (53.8)	51.0	.....	51.5–59.0 (54.1)
Thousandths of standard length				
Body depth	185–204 (195)	216	60	223–236 (230)
Caudal peduncle depth	105–117 (110)	137	67	146–154 (150)
Predorsal length	552–586 (570)	595	47	617–628 (623)
Prepelvic length	454–472 (464)	487	40	506–530 (521)
Depressed dorsal fin length	231–250 (239)	232	58	224–235 (227)
Sheath of anal fin (height along second ray)*	0–0 (0.0)	25	47	48–57 (53)
Head depth†	163–171 (168)	178	43	184–198 (191)
Head width	150–165 (157)	173	55	181–195 (186)
Interorbital width (least fleshy)	107–114 (111)	123	55	129–137 (133)
Snout length	93–101 (97)	98	25	97–108 (101)
Orbit length	73–78 (75)	73	60	70–71 (70)
Mouth width (over-all)*	89–94 (91)	98	35	111–111 (111)
Upper lip width (transverse)*	69–75 (72)	70	29	62–68 (65)
Nonimbricated scale on top of head (maximum diameter)*	46–59 (51)	62	50	69–75 (73)
Distance between pores on top of head (Pl. III)*				
Distance A	11–22 (15)	22	87	19–27 (23)
Distance B	46–54 (49)	56	33	67–75 (70)
Distance C	21–26 (24)	20	50	14–19 (16)
Character index: A + B − C	37–48 (40)	59	53	70–86 (76)
Ratio, head depth to orbit length	2.2–2.3 (2.3)	2.5	45	2.6–2.8 (2.7)

Angle, between dorsal and ventral contours of head‡	31–36 (33)	36	45	38–42 (39)
Displacement index§	235–249 (243)	251	25	269–281 (275)
Gillraker counts	5–7 (6.0)	8	49	9–12 (10.1)
Scale counts				
Head to caudal	44–51 (48.0)	41	53	34–36 (34.8)
Predorsal scales‖	25–29 (26.9)	22	71	18–22 (20.0)
Dorsal origin to anal origin	16–18 (17.4)	17	18	14–16 (15.2)
On top of head (total count)	23–30 (27.6)	24	42	18–20 (19.0)
Ray counts				
Dorsal	12–13 (12.4)	12	67	11–12 (11.8)
Anal	10–11 (10.6)	10	100	10–10 (10.0)
Caudal	16–16 (16.0)	17	26	19–20 (19.8)
Pectoral	17–18 (17.2)	18	73	18–19 (18.3)
Average hybrid index, measurements, ratios, etc.			48	
Counts			55	
Grand average			50	

* Measured with an ocular micrometer (7 units per mm.).

† Measured vertically, below posterior end of nonimbricated scale (Pl. III).

‡ Measured with an arm protractor.

§ 10,000 times the cube root of displacement of eviscerated body in cc., divided by standard length.

‖ All predorsal scales crossed by middorsal line to, but not including, the nonimbricated scale.

In general color tone (Pls. I and II) the hybrid is darker than the *F. d. diaphanus* associates, but lighter than the *F. heteroclitus macrolepidotus.* As a consequence the lateral dark bars (Pl. I) and the more or less crosswise blotching of the predorsal region (Pl. II) are of intermediate sharpness. In these features of coloration, however, there is an essential agreement with *F. d. diaphanus.* In the faint spotting of the sides, however, the approach is closer to the *heteroclitus* parental form. The blackish streak on the lower edge of the caudal peduncle in the hybrid is narrower than in *F. d. diaphanus* but broader than in *F. h. macrolepidotus.*

We regard this specimen as a very obvious hybrid, and as very consistently intermediate between its parental species.

Chriopeops goodei × *Lucania parva*

(Pls. IV–VI)

Field No. H41–12: 1 adult male (No. 1) found in a collection that was made in a shallow, diked lake and a small tributary stream, on the St. Marks Migratory Bird Refuge, just west of the Refuge fire tower, in Section 31 of Town 4 S., Range 2 E. The water here was fresh and rather clear, but brown-stained and with an organic taste. The bottom was of sand, with a little muck. *Naias marina* formed dense growths.

Field No. H41–15: 2 adult males (Nos. 2–3) obtained in a strongly brackish pond (open to the Gulf at very high tides), just east of the St. Marks Lighthouse, in Section 31 of Town 5 S., Range 1 E. The water was brown-stained, the bottom of muck and sand. The fish were seined out of reed beds filled with slimy algae.

Field No. H41–16: 1 adult female, collected along the shore of the Gulf of Mexico near the St. Marks Lighthouse, in Section 1 of Town 5 S., Range 1 W., close to the outlet of the brackish pond where collection H41–15 was made. Here the water was of almost normal Gulf salinity and moderately clear. The bottom was of soft to firm sand, with a slight growth of algae.

TABLE III

RELATIVE NUMBERS OF HYBRIDS, *Chriopeops goodei* × *Lucania parva*, AND OF THE PARENTAL SPECIES, IN COLLECTIONS FROM THE ST. MARKS MIGRATORY BIRD REFUGE, FLORIDA

	Chriopeops goodei	Hybrids	*Lucania parva*
Specimens in collection number			
H41–12	13	1 (male)	296
H41–13	......	......	1
H41–14	......	......	415
H41–15	18	2 (males)	344
H41–16	......	1 (female)	194
In collections containing hybrids	31 (3.6%)	4 (0.5%)	834 (96.0%)
In all 5 collections	31 (2.4%)	4 (0.3%)	1250 (97.3%)

These 3 collections, and 2 others, were made on April 7, 1941, on the St. Marks Migratory Bird Refuge of the Fish and Wildlife Service, with the consent and aid of Donald V. Gray, Refuge Keeper. The Refuge is on the

Gulf Coast in Wakulla County, western Florida. The area was formerly a salt marsh, but now most of the pond water is fresh because dikes have been built to keep out the sea water.

These hybrids occurred in company with an abundance of *Lucania parva* but with only a sparse population of *Chriopeops goodei* (Table III). The relative numbers for *Lucania* as given in the table are almost surely not higher, perhaps lower, than the true value for the population at the time of collection. Time did not permit picking all of the common species out of the net. The collections, however, were roughly random ones. The hybrids were not recognized in the field.

Except for Blue Springs near Marianna, farther inland, the St. Marks region is the westernmost locality from which we have collected *Chriopeops*. The 2 species have been taken together in the peninsular part of Florida, but an examination of the material fails to locate a single additional hybrid specimen. The hybrids were collected near the ecological as well as the geographical limit for *Chriopeops*, which is a more strictly fresh-water genus than *Lucania*.

When the collections listed above were sorted, the 4 *Chriopeops* × *Lucania* hybrids were separated readily from the specimens of the parental species. The features that first attracted attention lay in the distinctively intermediate coloration. How perfectly the pigmentary features of the hybrids interpose between those of the parental species is brought out by the illustrations (Pls. IV–VI) and by the following comparisons.

Lateral dark band on body.—In *Chriopeops* the intense band is composed of large expanded melanophores so closely placed that their processes overlap. On the anterior two-thirds of the body this evenly colored band is confined to the lower one-half to two-thirds of the axial scale row. As the scale row immediately below is usually almost free of melanophores except at the borders, the lower edge of the band is serrate, whereas the upper border is an even line. On the caudal peduncle the band covers the lower three-fourths of the axial scale row and also darkens the upper third of the scale row beneath, producing here a smooth border on both edges. The borders of the lower two-thirds of the scale pockets of the axial row are almost jet-black—very much more heavily pigmented than is the rest of the body.

In *Lucania* the very faint band is composed of only a few small melanophores, scattered over the middle part of the scales of the axial row, so as to produce a stippled appearance, but leaving the upper and lower ends of each scale more or less free of pigment. The melanophores approach a linear arrangement. The borders of these scale pockets are very slightly blackened, or no more heavily pigmented than is the rest of the body.

In the hybrids the band is of intermediate intensity, and the large melanophores which compose it are more sparingly placed than in *Chriopeops*, so that their processes are usually not in contact. As in *Chriopeops* the upper border of the band leaves the dorsal third of the scale light, but a few melanophores are developed here in the hybrids. The lower fifth of each axial scale pocket is either free of melanophores or much less densely stippled than elsewhere. On the caudal peduncle the melanophores of the band are mostly limited to the median half of each pocket, with a few scattered on the lower part of the pocket and on the upper fourth of the pocket below (as in *Chriopeops*). Anteriorly, the lower edge of the band shows a trace of the serration seen in *Chriopeops*. On the posterior part of the band the melanophores tend to be arranged in approximately linear rows. The borders of the axial scale pockets, especially on the dividing lines, are much more boldly blackened than in *Lucania* but less so than in *Chriopeops*.

CAUDAL SPOT.—In *Chriopeops* an intensification of melanophores on the scale pockets of the caudal base produces a jet-black spot, which varies considerably in size: its width may be slightly less than the height of 1 scale, or equal to the height of 2 scales. The spot extends from the end of the hypural plate to about the end of the scaly area on the base of the caudal. It is most intense in its anterior half; the pigmentation becomes more or less diffuse posteriorly.

In *Lucania* the lateral band ends at the hypural plate, and the scale pockets on the base of the caudal are entirely devoid of pigment except on their borders.

In the hybrids the intermediate lateral band extends to the end of the scaly sheath at the base of the caudal, widening posteriorly to the hypural plate. In 2 specimens there is an intensification of pigment on the first scale pocket behind the hypural, but in the other 2 the pigmentation here about equals that on the rest of the band.

BAND ON OPERCLE.—In *Chriopeops* the dark lateral band continues nearly straight forward to the orbit. Across the opercle it maintains the same intensity and width as on the body. Between the preopercle and the orbit the pigmentation becomes less intense and the melanophores are smaller.

In *Lucania* the very indistinct band continues forward on the opercle, bending down to meet the middle of the eye.

In the hybrids the band is of intermediate intensity and bends down slightly to meet the eye, but less so than in *Lucania*. On the opercle the intensity of the pigment is slightly greater than on the body band, but this region is less blackened than it is in *Chriopeops*.

BAND AROUND MUZZLE.—In *Chriopeops* deep-lying pigment produces a band which extends forward from the eye and around the chin, but is not developed on the lips. This mark is about as wide as the pupil. Overlying the band there is a scattering of small melanophores which extend beyond the dorsal edge of the stripe, but rarely occur below its ventral edge.

In *Lucania* a few deep-set melanophores form a suggestion of a stripe from the eye to the mouth, but there is no trace of a band on the chin. Small superficial melanophores cover the same area between the eye and the mouth, but on the chin extend downward and backward to form a shaded area about twice as wide as the band on the mandible of *Chriopeops.*

In the hybrids the deep-lying band of pigment is situated as in *Chriopeops,* but is less intense. The overlying melanophores are fewer than in *Lucania;* they extend below the band, but not so far downward and backward as in that genus.

PREDORSAL STRIPE (Pl. VI).—In *Chriopeops* there is no trace of a predorsal stripe; the scales along the middorsal line resemble the adjoining ones in pigmentation. In *Lucania* a definite predorsal stripe extends from the origin of the dorsal fin to the occiput. In the hybrids a predorsal stripe occurs, but it is neither so wide nor so intense as it is in *Lucania.*

LIGHT STRIPE ABOVE LATERAL BAND.—In *Chriopeops* a light band 2 scale rows high lies just over the dark lateral band. In the light zone the outlines of the scale pockets are more narrowly pigmented than elsewhere, and the melanophores over the pockets are much sparser than on the scales above and below. In *Lucania* the scale pockets are evenly pigmented above the faint lateral band. In the hybrids the light band is apparent and follows the pattern of *Chriopeops,* but is not so sharply delineated as in that genus.

DARK BAND ALONG ANAL BASE.—In *Chriopeops* an intense band of black pigment extends from just above the anus to the base of the caudal fin, skirting the anal base and following the mid-ventral line of the caudal peduncle. It is composed of deep-lying expanded melanophores, so closely placed that their processes are usually in contact. The bands are about one-half a scale row wide on the sides of the anal fin, and join back of the fin to cover the mid-line row of scales and the lower portions of adjacent scale pockets. In the mid-line between the anal and caudal fins there is also a conspicuous row of small melanophores, closely spaced in a single file. The borders of the band are relatively definite in the anterior portion, but become less well-defined on the caudal peduncle.

In *Lucania* there is no trace of a band along the anus and anal fin, but from the anal fin to the base of the caudal there is a row of small deep-lying melanophores, as in *Chriopeops.*

In the hybrids the bands are present, but the melanophores are more widely separated than in *Chriopeops,* so that their processes usually do not touch. The width of the band between the anal and caudal fins is about one-half to three-fourths the width of the median line of scales.

PIGMENTATION OF SCALE BORDERS BELOW AXIAL ROW.—In *Chriopeops* the pockets of the scale rows below the axial series tend to be weakly outlined by melanophores. The lower half of the first row below has a more or less well-defined edging of small or large melanophores. The second series below has the margins of the pockets poorly set off by small melanophores, or not at all pigmented, and the third row below is not outlined.

In *Lucania* the margins of the scale pockets below the lateral band are well defined. The first row beneath the axial one is sharply delineated by 2 or more marginal rows of large melanophores. The second series is always outlined by at least 1 row of large black color cells. The third row below is usually set off by a single file of well-developed melanophores, but in some specimens these are lacking. The fourth row is either poorly outlined or unpigmented.

In the hybrids the first row below the axial series has the scale pockets sharply delineated by 1 or 2 rows of large melanophores. This margining is more marked than in *Chriopeops,* but is weaker than in *Lucania.* The scale-pocket edgings on the second scale series below are weakly defined, and the third row is unpigmented.

SCALES ON TOP OF HEAD (Pl. VI).—In *Chriopeops* the scales on the top of the head are evenly pigmented, so that the borders are not sharply delineated. In *Lucania* the melanophores are concentrated toward the borders of these scales and tend to leave a light area in the center, so that the outlines stand out sharply. In the hybrids the melanophores are concentrated toward the scale borders, but the clear central area is less depigmented than in *Lucania.* The appearance is therefore intermediate.

INTERNARIAL PIGMENT SPOT (Pl. VI).—In *Chriopeops* there is a roughly triangular spot of deep-lying melanophores just in front of each posterior nostril. The base of the triangle lies between the posterior nostril and the posterior nasal pore of the lateral line system, and the apex lies between the anterior nostril and the anterior nasal pore. The melanophores are large, and their processes are in contact. In *Lucania* this spot is indistinct and contains only a few widely separated melanophores. In the hybrids it is well defined, but the melanophores are more widely spaced than they are in *Chriopeops.* Again the appearance is intermediate.

PIGMENT BELOW EYE.—In *Chriopeops* the cheek below the eye is either wholly unpigmented, or, in some males, bears a few small scattered melanophores. There is almost no tendency for a concentration of pigment along the lower border of the orbit.

In *Lucania* this region is rather well covered with large melanophores which tend to form a subocular bar, and posteriorly to line the scale pockets. There is always a concentration of pigment along the lower orbital border. In the young, in females, and in salt water there is a tendency for this pigmentation to be weaker.

In the hybrids there is more pigment below the eye than in *Chriopeops* but less than in *Lucania.* Most of the melanophores that do occur in this area are located along the lower border of the orbit.

PIGMENTATION ON BASE OF PECTORAL FIN.—In *Chriopeops* the pectoral base is either unpigmented or bears only a few small scattered melanophores. In *Lucania* there are several to many, generally large melanophores; usually enough to darken this area. Dorsally, some of these black pigment cells often line the scale pockets. In the hybrids the pectoral base has a few rather small melanophores sometimes tending to form borders on the scale pockets.

Pigmentation of eye.—In *Chriopeops* the whole upper three-fourths of the eye is darkened, with more or less intensification in line with the lateral band. Ventrally, the dark color occurs next to the pupil, leaving a silvery crescent around the lower border of the eye (including the area where the eye of *Lucania* is crossed by a dark bar).

In *Lucania* the most prominent mark is a broad blackish bar, rarely indistinct, which extends across the cornea downward and slightly forward from the pupil. There is a similar but weaker band running upward and backward from the pupil and usually another, narrower one, extending upward and forward from the pupil. Either one or the other of the dorsal bands may be weak, and they may be scarcely evident when the whole upper part of the cornea is darkened. Commonly, there is a tendency for a silvery ring to form around the inner rim of the cornea. The area between the ventral and the posterolateral corneal bars is silvery; this silvery area may extend to the pupil, or may be separated from it by dark pigmentation.

The hybrids tend to combine the color markings of the parental species. Almost the entire cornea is blackish, becoming silvery posteroventrally. This silvery crescent is intermediate in position. The ventral dark bar of *Lucania* is rather poorly developed and hardly distinct from the dark area on either side of it; furthermore, the silvery area tends to extend into or below the position of the ventral bar. The whole upper part of the eye is so dark that only bare traces can be seen of the 2 upper dark bars of *Lucania,* and these dark bars if developed seem to be fused with the posterior and anterior darkened areas that form part of the lateral band in *Chriopeops.* There is a dark area on the cornea around the entire pupil, with a tendency for a silvery ring to form at the extreme pupillary margin.

Pigmentation of dorsal fin in males.—In *Chriopeops* the basal one-third to one-half of each interradial membrane is jet-black. Rays 1, 2, and 3 are overlain with melanophores in this basal area, but the other rays are relatively free of melanin. The border of the fin is diffusely pigmented on the interradial membranes; this color becomes intensified to black at the extreme margin.

In *Lucania* a jet-black blotch is located on the basal one-half of the front part of the dorsal fin, from the first to the third ray. This prominent mark covers the rays as well as the membranes. Distally, the pigmentation becomes somewhat less dense and is confined to the interradial membranes. The pattern of the rest of the fin is much like that of *Chriopeops,* except in that the subbasal area is dusky instead of jet-black, and the black border is less intense.

In the hybrids there is a blotch on the front of the dorsal fin, as in *Lucania,* but this mark is continued backward on the interradial membrane between the third and fourth rays. In the intensity of pigment over the rest of the fin the hybrids are intermediate between *Chriopeops* and *Lucania.*

PIGMENTATION OF ANAL FIN IN MALES.—In *Chriopeops* the basal one-third of the interradial membranes behind ray 4 varies from dusky to jet-black; this part of the membrane between rays 1 and 3 carries no pigment, and between rays 3 and 4 the membrane is lightly pigmented. There is a narrow black band on the border of the fin, but between the 2 dark areas the membranes are unpigmented.

In *Lucania* melanophores are very sparsely distributed over all the membranes, except on the basal portions between rays 1 and 3. There is a black band on the border as in *Chriopeops*.

In the hybrids the situation is the same as in *Chriopeops*, except that the dark basal areas are much less deeply and more sparsely pigmented than in all except a few specimens of *Chriopeops*.

PIGMENTED BORDER ON THE CAUDAL FIN OF MALES.—In *Chriopeops* there is no trace of a pigmented border on the end of the caudal fin. In *Lucania*, however, the caudal has a diffusely pigmented dark border, which is composed of small scattered melanophores on the membranes and rays, and which covers approximately the posterior one-third of the fin. In the hybrids this band is developed rather weakly in one male, very slightly in another, and not at all in the third.

COLOR ON CAUDAL FIN OF MALES.—In life there is a red blotch on the basal part of each caudal lobe in *Chriopeops*. *Lucania* lacks these blotches. One of the male hybrids showed a trace of these red marks, despite the fading due to preservation for some weeks in alcohol.

OTHER CHARACTERS IN THE *Chriopeops* × *Lucania* CROSS.—In *Chriopeops* the upper and lower corners of the caudal fin are more broadly rounded than they are in *Lucania*, but the posterior edge is more abruptly truncate. In *Lucania* the posterior border of this fin forms a more regular arc. The hybrids are again intermediate (Pls. IV–V).

In *Chriopeops* the muzzle is slenderer than in *Lucania*, and the mandible is less massive and less prominent. In this respect, as in others, the hybrids are less extreme than is either parental species (Pls. IV–V).

Perhaps the most interesting character in which the hybrids are intermediate is the dentition. As its chief known generic criterion, *Chriopeops* has a regular inner row of small teeth, in addition to an outer file of large, strong teeth. In *Lucania* the teeth are strictly uniserial. The hybrids show, behind the main row, a few very small teeth obviously representing the inner row of *Chriopeops*. This is hardly the expected arrangement for a species, and is a very strong indication of hybridization.

The local race of *Chriopeops goodei* involved in the hybridization under study is a much slenderer fish than the type of *Lucania* in the same region (in some localities the local type of *Chriopeops* is as robust as the associated *Lucania*). The intermediacy of the hybrid in the depth of the body and of the caudal peduncle is brought out in Table IV. A similar situation applies to certain other proportions, as indicated in this table and in Plates IV–VI.

TABLE IV

COMPARISON OF THE *Chriopeops* × *Lucania* HYBRIDS WITH THE PARENTAL SPECIES

		Chriopeops goodei (P_1)	Hybrids					*Lucania parva* (P_2)	Difference Between Averages	Per Cent Difference†	Hybrid Index				
		(12 Males, 3–8 Females)*	Males				Female	(15 Males, 10 Females)			Males			Female	Average
		Range (Average)	No. 1	No. 2	No. 3	Average		Range (Average)			No. 1	No. 2	No. 3		
Standard length,‡ mm.	Males	25.4–31.6 (27.9)	24.9	28.0	28.7	(27.2)	......	25.6–29.3 (27.5)	0.4	......	......	......	......	......	......
	Females	30.7–33.9 (32.0)	......	......	......	......	33.5	30.5–33.1 (32.3)	0.3	......	......	......	......	......	......
$\frac{1000\ \text{T.L.}}{\text{S.L.}} - 1000$§	Males	206–237 (222)	253	246	244	(248)	......	241–282 (261)	39	18	79	56	51	......	64
	Females	173–200 (184)	......	......	......	......	248	250–297 (275)	91	49	......	......	......	70	
Thousandths of standard length															
Body depth	Males	197–240 (219)	237	257	251	(248)	......	255–303 (285)	66	30	51	58	48	......	47
	Females	195–222 (212)	......	......	......	......	236	278–306 (289)	77	36	......	......	......	31	
Caudal peduncle depth	Males	117–138 (130)	141	150	146	(146)	......	154–177 (165)	35	27	31	57	46	......	43
	Females	109–114 (111)	......	......	......	......	131	152–174 (164)	53	48	......	......	......	38	
Head length	Males	284–297 (289)	301	300	303	(301)	......	298–335 (312)	23	8	52	48	61	......	48
	Females	260–289 (273)	......	......	......	......	278	280–296 (289)	16	6	......	......	......	31	
Head width	Males	136–154 (144)	157	161	157	(158)	......	160–180 (169)	25	17	52	68	52	......	51
	Females	139–146 (142)	......	......	......	......	146	151–168 (155)	13	9	......	......	......	31	
Eye length	Males	88–98 (92)	100	96	96	(97)	......	96–109 (102)	10	11	80	40	40	......	49
	Females	78–86 (82)	......	......	......	......	89	97–105 (101)	19	23	......	......	......	37	
Pelvic fin length	Males	150–173 (162)	149	146	160	(152)	......	127–164 (146)	16	10	81	100	13	......	55
	Females	118–137 (126)	......	......	......	......	131	137–157 (146)	20	16	......	......	......	25	
Pectoral fin length	Males	172–181 (176)	197	186	192	(192)	......	191–213 (202)	26	15	81	39	88	......	63
	Females	156–171 (161)	......	......	......	......	176	177–206 (195)	34	21	......	......	......	44	
Counts															
Dorsal rays		9–12 (10.25)	12	11	11	(11.25)‖	11	11–13 (11.36)	1.11	11	158	68	68	68	90
Pectoral rays (both fins)		23–27 (24.50)	26	26	26	(26.0)	26	24–30 (26.88)	2.38	10	63	63	63	63	63
Scales, head to caudal		29–30 (29.55)	28	28	28	(28.0)	28	25–27 (26.24)	3.31	11	47	47	47	47	47
Scales, around body¶		18–20 (19.65)	20	22	20	(20.5)	20	20–23 (21.96)	2.31	12	15	102	15	15	37
								Averages (measurements)	35	22	63	58	50	38	52
								Averages (counts)	2.28	11	71	70	48	48	59
								Grand averages	......	......	66	62	49	42	55

* 3 females measured, 8 counted.
† As compared with the figure for *Chriopeops goodei.*
‡ Specimens of parental species were selected to match the hybrids in size (for the measurements).
§ Relation between total length and standard length.
‖ Averages for counts apply to both sexes.
¶ Counted from first scale in front of dorsal downward and forward to mid-line of belly, then upward and backward to the point of origin.

The interjacency of the hybrids between the local stocks of the parental species is also indicated by the counts of dorsal and pectoral rays and of scales (Table IV), despite the fact that the meristic differences between the parents are slight. The average hybrid index for the measurements is 52, for the counts, 59, for both measurements and counts, 55—a deviation of only 10 per cent from an indication of exact intermediacy.

When all this cumulative evidence is considered, it can hardly be questioned that *Chriopeops goodei* and *Lucania parva* occasionally hybridize, to produce offspring that are wonderfully intermediate between the parental species.

Fundulus sciadicus × *Plancterus kansae*

U.M.M.Z. No. 135141: 1 half-grown fish, collected by Raymond E. Johnson and Paul Romberg in the South Platte River, 2 miles northeast of the Colorado state line, in Deuel County, Nebraska, on July 21, 1940.

TABLE V

RELATIVE NUMBERS OF HYBRIDS, *Fundulus sciadicus* × *Plancterus kansae*, AND OF THE PARENTAL SPECIES

Specimens from the Platte Rivers (Excluding Tributaries)	*Fundulus sciadicus*	Hybrids	*Placterus kansae*
Specimens in random collection No. 266	4 (0.7%)	1 (0.2%)	554 (99.1%)
In collections (some not random) containing both species	291 (27.4%)	1 (0.1%)	769 (72.5%)
In all Platte River collections	301 (16.7%)	1 (0.1%)	1501 (83.3%)

When this hybrid was collected the South Platte River was reduced to a series of isolated pools in a broad, coarse-gravelled bed. Each pool was about 20 feet in diameter and 2 feet deep, with a bottom of fine sand and soft muck. There was a slight current caused by a subsurface flow between the pools. The relative coolness of the water (76° F.) was due in part to that factor and in part to the deep shade furnished by a very thick mat of *Spirogyra* and by cottonwood and willow trees growing on the higher gravel ridges in the stream course.

An attempt was made to seine and preserve in formalin every fish present in 1 pool. In addition to the 1 hybrid, 23.4 mm. in standard length, there were obtained 4 examples of *Fundulus sciadicus* 20 to 29 mm. long, 554 specimens of *Plancterus kansae* 16 to 61 mm. long, 4 thriving young of *Pimephales promelas promelas*, and 2 emaciated adults of *Notropis dorsalis: dorsalis* × *piptolepis*. In Nebraska *Fundulus sciadicus* occurs mainly north of the Platte River and *Plancterus kansae* south of that river. The 2 genera meet and occur together only in the North and South Platte rivers proper. *Fundulus sciadicus* was very seldom found in abundance at any point in those streams, and was greatly outnumbered by *Plancterus kansae* at most places where the 2 species were seined together. A careful check of all the Platte River material failed to uncover another hybrid.

The systematic and nomenclatorial status of these cyprinodonts, the only species of the family known from Nebraska, has been treated by Hubbs (1926: 10–11, 15).

Despite its small size the peculiar cyprinodont specimen from the Platte River displays characters by which it is readily diagnosed as an interspecific hybrid. Attention was first attracted to this fish when it was seen to combine the vertical bars of *Plancterus kansae* with the rather uniform brassy to reddish olive undertone, overlain with minute brownish flecks, of *Fundulus sciadicus*. *Plancterus kansae* contrasts sharply with *Fundulus sciadicus* in being greenish above, yellowish on the sides, and almost white below. Its sides are crossed by 12 to 18 prominent vertical dark bars, extending from the head to the caudal fin. The Plains species of *Fundulus* never has a trace of these cross bars. The hybrid shows 8 to 10 of the cross bands, almost hidden by the uniform dark undertone.

A closer scrutiny of the pigment disclosed other, equally distinctive features of the parental species, which are blended in the hybrid. In *Fundulus sciadicus* small melanophores are very evenly dusted over the dorsal and lateral scales and form a pattern of fine pepper marks around the front of the muzzle. In *Plancterus kansae* the melanophores are larger. Those on the back and upper sides are loosely clumped into pigment spots that tend to be concentrated on the posterior field of each dorsal and lateral scale, leaving the intervening spaces clear. Around the muzzle the melanophores are larger and less densely spaced. In these characters the hybrid is intermediate, possibly favoring the *Plancterus* parent in the pigmentation of the dorsal scales. The *Fundulus* displays a prominent middorsal dusky stripe, which extends with uniform intensity from the occiput to the dorsal fin. In *Plancterus* the predorsal stripe is restricted to a distinct black diamond-shaped spot just in advance of the origin of the dorsal. The hybrid possesses a diamond-shaped mark at the same point, but this spot is elongated into a stripe extending halfway to the occiput.

The *Fundulus* has very little pigment on the interradial membranes of the pectoral fin. Only a few minute flecks show faintly near the fin margin, but elongated melanophores in 1 series form a sharp, black outline along the full length of the upper and lower border of each ray. In *Plancterus*, on the other hand, the pigment cells are scattered widely over the membranes and do not form a definite border along the sides of the rays. The hybrid resembles the *Fundulus* parent in having each pectoral ray outlined with black, but the pigmentation is very weak and in places the outline is broken.

In view of the wide differences between the presumed parental species in the number of scales and fin rays, the supposition that the aberrant species is a hybrid was tested by counting the meristic features. Four ray counts and 4 scale counts were taken on the hybrid and on 15 specimens of each parental species. As was expected the counts proved to be almost exactly intermediate (Table VI, lower part). Each value for the hybrid intervened between the corresponding counts for the parental species, when the figures were widely divergent, and agreed with the adjacent extreme count of the one or the other type, when the ranges for the parents were barely separated.

Since all of the scale enumerations and all of the fin counts except the caudal ray number were found to be definitely higher in the *Plancterus* than in the *Fundulus,* it is obvious that the distinction between the species can be emphasized by a character index: the number of caudal rays subtracted from the sum of all 7 other counts for each individual. A gap of 97 points separates the index values for the 2 species, and the figure for the hybrid lies nearly midway between:

Character index for scale and ray counts:

Fundulus sciadicus	127 to 141 (average, 132)
Hybrid	173
Plancterus kansae	221 to 241 (average, 229)

The hybrid index for this character index is 42.

Similar intermediacy is also shown for the critical measurements (Table VI, upper part). In several proportions the approach is greater toward the *Plancterus* than toward the *Fundulus* parent. The average hybrid index of 62 for the measurements, however, very strongly confirms the identification of the specimen as a hybrid. The grand average hybrid index of 55, for all counts and measurements, is only 10 per cent higher than the value of exact intermediacy, and happens to be identical with the similar value obtained for the *Chriopeops* × *Lucania* crosses.

The position of the dorsal fin is brought out not only by the proportionate measurement of predorsal length (Table VI) but also by the relation of the dorsal to the anal fin. In *Fundulus sciadicus* the front end of the dorsal fin lies distinctly behind the origin of the anal fin. *Plancterus* has the insertion of the dorsal fin ahead of the anal origin. In the intermediate hybrid the origins of the 2 fins are on about the same vertical.

The caudal fin of the hybrid shows an intermediate feature in the curvature of the posterior border, which is neither as rounded as in the *Fundulus* nor so truncate as in *Plancterus.* Probably because of its youth or its condition of preservation, the hybrid does not display interjacency in the degree of fanning out of the caudal fin (which is greater in the *Fundulus* than in the *Plancterus*).

The hybrid also intervenes between the parental forms in the position and structure of the pelvic fins. In this respect the 2 species are quite distinct. In the *Fundulus* the pelvics are inserted very close together and are conjoined at their bases by a large sheath of skin. The *Plancterus* pelvics are separated by a distance almost as great as the width of the base of either fin, and no dermal fold binds their proximal halves together.

The gill-rakers also proclaim the specimen to be a hybrid. In *Fundulus sciadicus* these structures are short and on the lower part of the arch become rudimentary tubercles. Even anteriorly *Plancterus* has long, slender, and curved rakers. In the hybrid they are rather long, but on the lower part of the arch are definitely shorter, almost knoblike.

TABLE VI

COMPARISON OF THE *Fundulus* × *Plancterus* HYBRID WITH THE PARENTAL SPECIES

	Fundulus sciadicus	Hybrid		*Plancterus kansae*
	(10 Measured, 15 Counted) Range (Average)	Proportions and Counts	Hybrid Index	(10 Measured, 15 Counted) Range (Average)
Standard length, mm.*	20.1–29.1 (24.5)	23.4	......	22.0–24.1 (23.1)
Thousandths of standard length				
Predorsal length	670–700 (688)	674	21	590–643 (622)
Body depth	231–261 (250)	218	68	195–213 (203)
Caudal peduncle depth	149–168 (158)	128	68	109–123 (114)
Head depth	189–203 (196)	184	86	177–188 (182)
Snout length	97–107 (103)	107	80	100–111 (108)
Eye length	75–94 (87)	81	50	70–77 (75)
Anal fin, depressed length	219–258 (237)	222	58	204–230 (211)
Counts				
Dorsal rays	9–10 (9.1)	12	51	14–16 (14.8)
Anal rays	11–12 (11.5)	13	68	13–15 (13.7)
Principal caudal rays	16–19 (17.9)	16	49	13–15 (14.0)
Pectoral rays	14–15 (14.2)	15	40	16–17 (16.2)
Scales, lateral line	33–40 (35.9)	47	39	59–69 (64.5)
Scales, around body†	30–36 (32.6)	43	38	57–66 (60.3)
Scales, around peduncle	18–21 (19.8)	25	51	28–33 (30.0)
Scales, predorsal	21–25 (23.1)	30	64	36–43 (39.9)

Average hybrid index		
Average hybrid index,	measurements	62
	counts	50
	Grand average	55

* Specimens of the parental species were selected to match the hybrid in size.
† Counted vertically around body just in front of the pelvic fins, in a zigzag line.

The hybrid is interposed between *Fundulus* and *Plancterus* in at least one of the generic differences (Hubbs, 1926: 14). In *Fundulus* the gill-opening is free to the angle of the opercle, and the slit therefore extends far above the upper end of the pectoral base. In *Plancterus* the upper part of the opercle is bound down to the shoulder girdle, so that the slit extends upward only to about opposite the uppermost pectoral ray. The hybrid is nicely intermediate. The other assigned generic differences (length and convolution of the intestine, and the strength and shape of the pharyngeal teeth) were not investigated, because of the small size of the one hybrid specimen.

CONCLUSIONS

The family Cyprinodontidae is now added to the list of fish groups in which species are known to hybridize in nature. We write "known to hybridize" advisedly, because in our judgment the circumstantial evidence for natural hybridization in this family, in conjunction with the data for other groups, is reliable and conclusive. The basis for interpreting certain specimens as interspecific hybrids has recently been outlined (Hubbs, Hubbs, and Johnson, 1943: 11–14), and need not be repeated here.

The three cyprinodontid hybrids now described lie between *Fundulus diaphanus diaphanus* and *Fundulus heteroclitus macrolepidotus,* between *Chriopeops goodei* and *Lucania parva,* and between *Fundulus sciadicus* and *Plancterus kansae. Fundulus heteroclitus macrolepidotus* is chiefly marine; the race of *Lucania* involved ranges from salt to fresh water; the other species are fresh-water fishes. The principle that fresh-water forms are more subject to hybridization than are marine fishes is thus extended.

Natural hybridization between species in the Cyprinodontidae must be one of the rarest of phenomena. The discovery would not likely have been made, had it not become a custom in ichthyological field work to collect and preserve large series of specimens.

As usual the new fish hybrids prove more or less exactly intermediate between the parental forms, in the various characters by which the species are distinguishable. The features in which the cross-bred individuals intervene between the parental species are deep-seated as well as superficial. In two of the three combinations there are involved structures so trenchantly different as to be regarded as of generic significance, along with details of pigmentation. The *Fundulus* × *Fundulus* hybrid exhibits intermediacy in such features as the pattern formed by the pores and canals on the top of the head, the height of the anal sheath at the side of the oviducal pouch, and the volume of the eviscerated body. There are differences, too, in meristic counts and in measurements. In brief, the hybrids are intermediate in the whole complex that makes up the "different reaction systems" of the parental species. On the percentage scale in which the values for the characters of one parental species are set at 0 and that for the other species at 100, the degree of intermediacy of the three hybrids under discussion is expressed by the grand average hybrid index of 50 for the cross between species retained in *Fundulus* and 55 for each of the crosses regarded as intergeneric.

The three cyprinodont hybrid combinations agree in another respect, namely, that the only known hybrids have been taken where only a few fish of the one parental species occur with an abundance of the other parent (Tables I, III, and V). That the association of a rare and an abundant species is conducive to hybridization has been indicated a number of times. Most of the pertinent evidence has not been published, but two outstanding cases of this phenomenon were discussed in the recent paper on catostomid hybrids (Hubbs, Hubbs, and Johnson, 1943: 39, 48). Apparently, the very rare species may often or even usually be forced to miscegenate with the abundant species.

If most interspecific hybridization leads to sterility and hence to biotic inefficiency, as we assume it does, the tendency of species to hybridize where they are rare may well be an adverse factor in survival in the periphery of their ranges, and hence in their dispersal. Local extirpation of the rare form might thus be accelerated, as Greene (1935: 87–89) has suggested for local populations of the cyprinid fish *Clinostomus elongatus*. Ranges might thus be limited, and complementary distributions of related species built up and maintained. If, as is now generally conceded, a small advantage may lead to the spread of a gene through a population, it must be granted that a small loss in biotic potential, induced through occasional hybridization, might in time have a deleterious effect in dispersal and survival. We assume, however, that hybridization would ordinarily be only a contributing factor in limiting the range of a species or the life of a local population.

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PLATE I

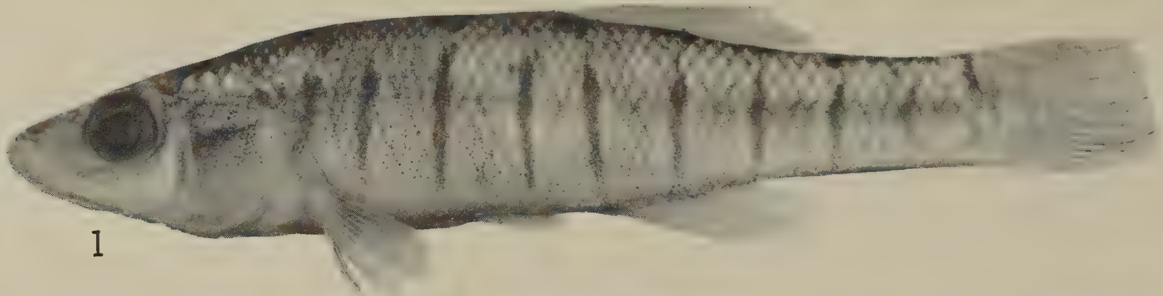
Hybrid cyprinodont, *Fundulus diaphanus diaphanus* × *Fundulus heteroclitus macrolepidotus*, and the parental species

All from Lake of Shining Waters, Prince Edward Island, Gulf of St. Lawrence. Photographed to the same scale by Clarence Flaten.

FIG. 1. *Fundulus diaphanus diaphanus,* female.

FIG. 2. Female hybrid, 51 mm. in standard length.

FIG. 3. *Fundulus heteroclitus macrolepidotus,* female.



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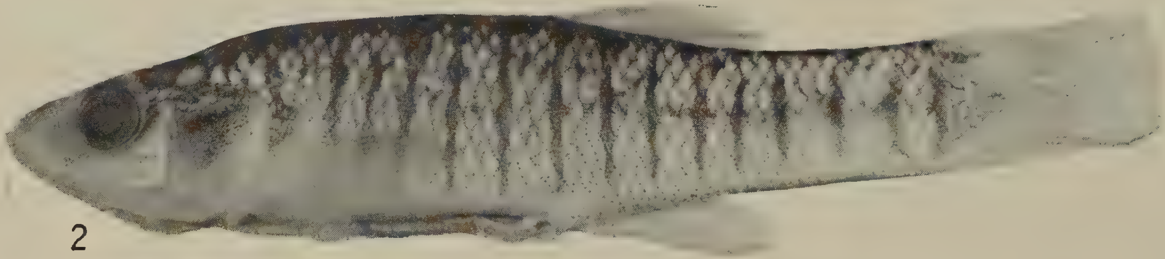

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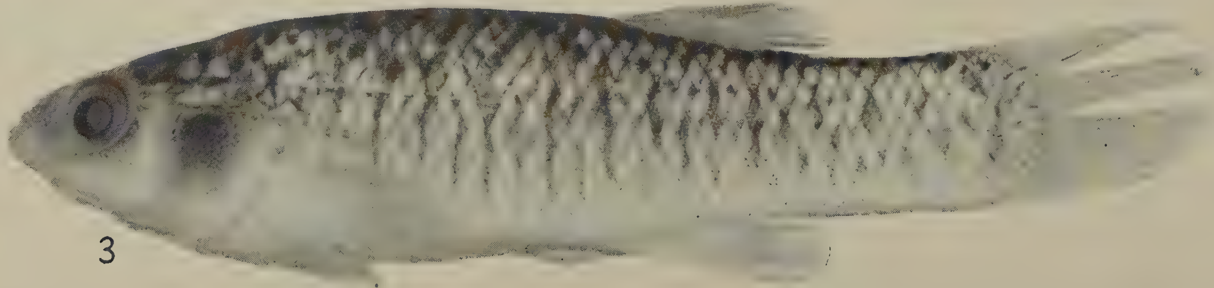

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PLATE II

Dorsal view of hybrid cyprinodont, *Fundulus diaphanus diaphanus* × *Fundulus heteroclitus macrolepidotus*, and of the parental species

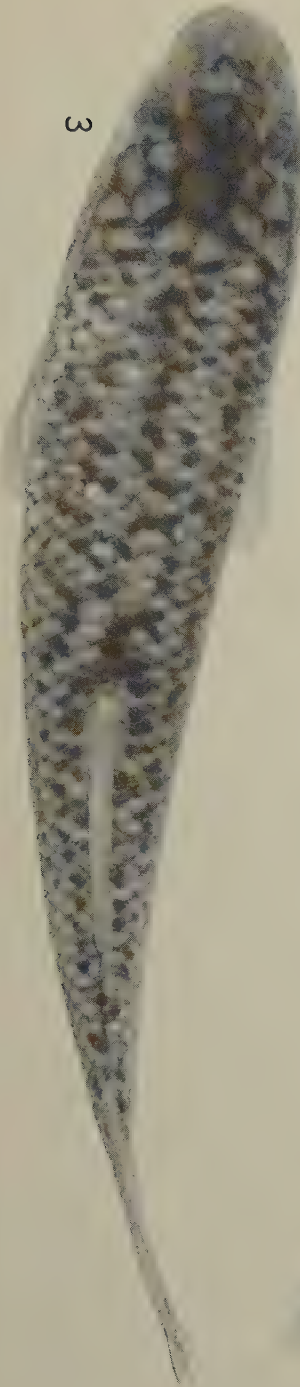
All from Lake of Shining Waters, Prince Edward Island, Gulf of St. Lawrence. Same specimens as shown in Plate I. Photographed to the same scale by Clarence Flaten.

Fig. 1. *Fundulus diaphanus diaphanus*, female.

Fig. 2. Female hybrid, 51 mm. in standard length.

Fig. 3. *Fundulus heteroclitus macrolepidotus*, female.

Plate II



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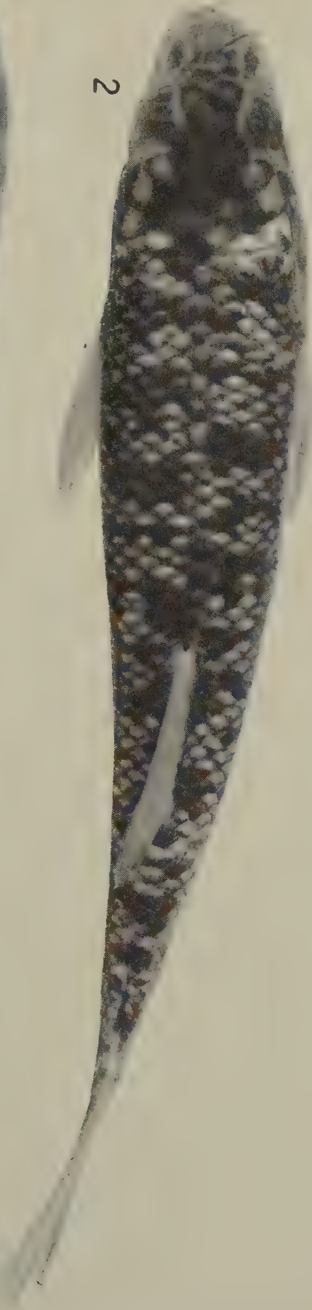

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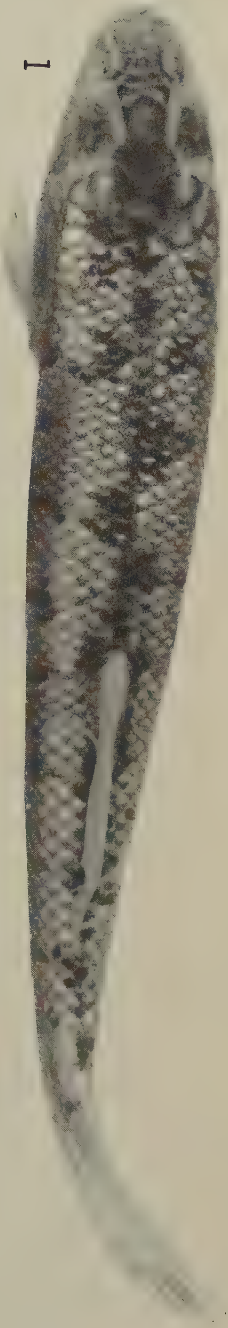

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PLATE III

Dorsal view of head of hybrid cyprinodont, *Fundulus diaphanus diaphanus* × *Fundulus heteroclitus macrolepidotus,* and of the parental species

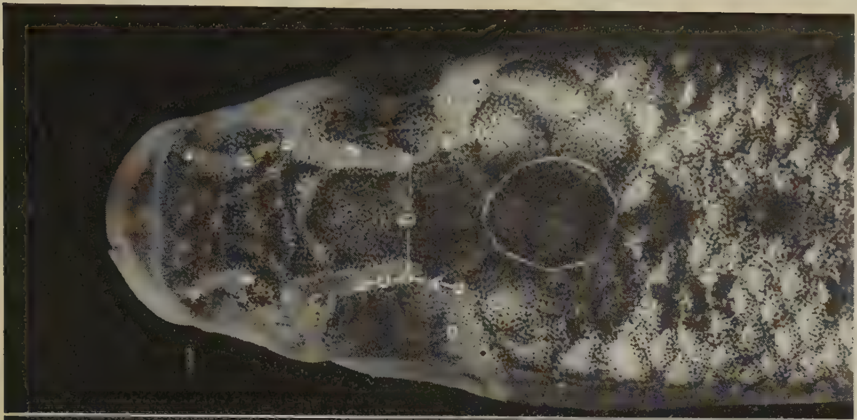
All from Lake of Shining Waters, Prince Edward Island, Gulf of St. Lawrence. Same specimens as shown in Plates I and II. Photographed to the same scale by Clarence Flaten; retouched to emphasize sensory pores and the nonimbricated scale. A, B, C are distances measured by eye-piece micrometer and used in computing a character index.

Fig. 1. *Fundulus diaphanus diaphanus,* female.

Fig. 2. Female hybrid, 51 mm. in standard length.

Fig. 3. *Fundulus heteroclitus macrolepidotus,* female.

PLATE III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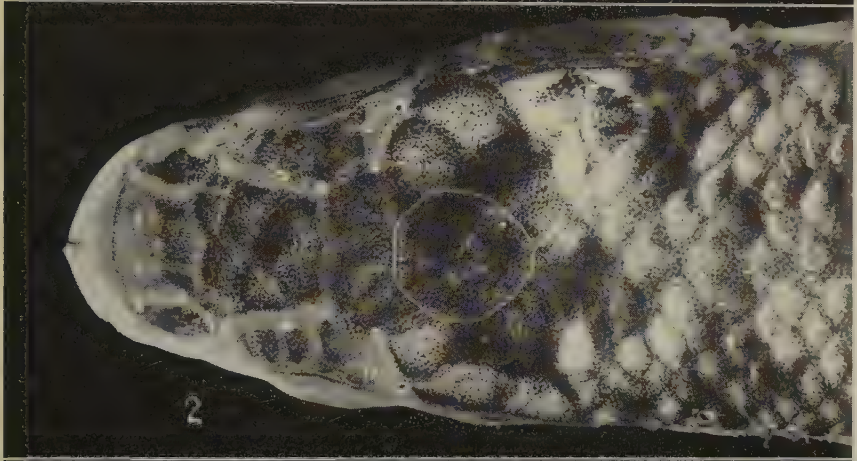
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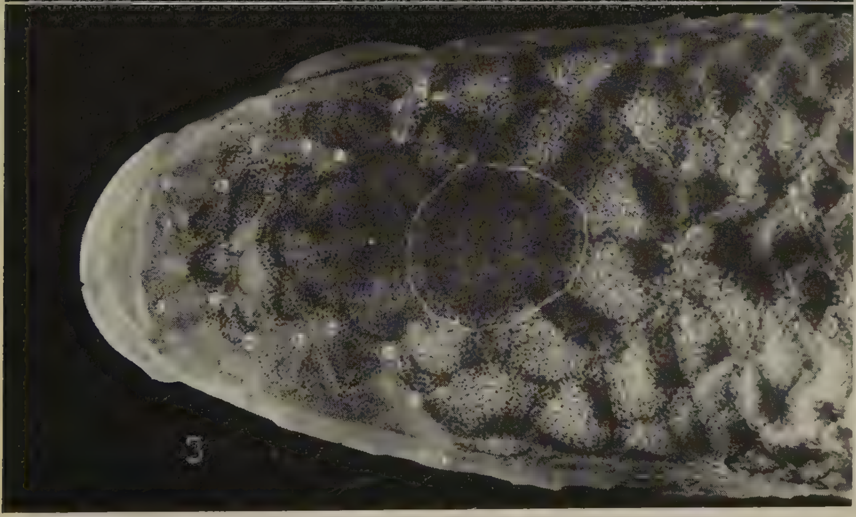
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PLATE IV

Hybrid cyprinodont, *Chriopeops goodei* × *Lucania parva,* and the parental species

All from St. Marks Migratory Bird Refuge, Florida. Photographed to the same scale by Clarence Flaten.

FIG. 1. *Lucania parva,* male, from collection H41–15.

FIG. 2. Male hybrid, 28.7 mm. in standard length, from same collection.

FIG. 3. *Chriopeops goodei,* male, from same collection.

PLATE IV

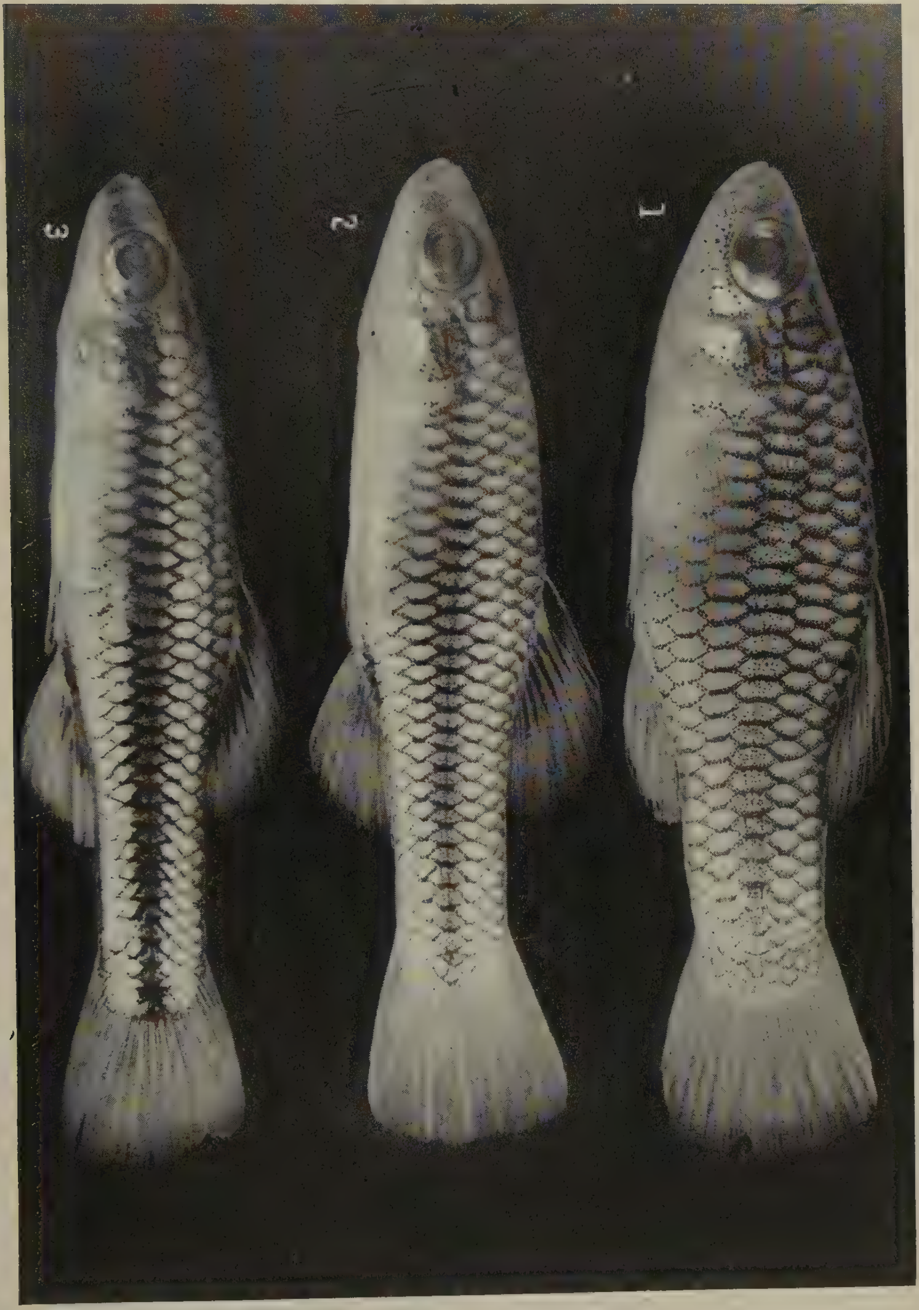

PLATE V

Hybrid cyprinodont, *Chriopeops goodei* × *Lucania parva,* and the parental species

All from St. Marks Migratory Bird Refuge, Florida. Photographed to the same scale by Clarence Flaten. Owing to the bright illumination required in taking this picture, the melanophores on the lower part of the head are scarcely apparent.

Fig. 1. *Lucania parva,* female, from collection H41–16.

Fig. 2. Female hybrid, 33.5 mm. long, from same collection.

Fig. 3. *Chriopeops goodei,* female, from collection H41–15.

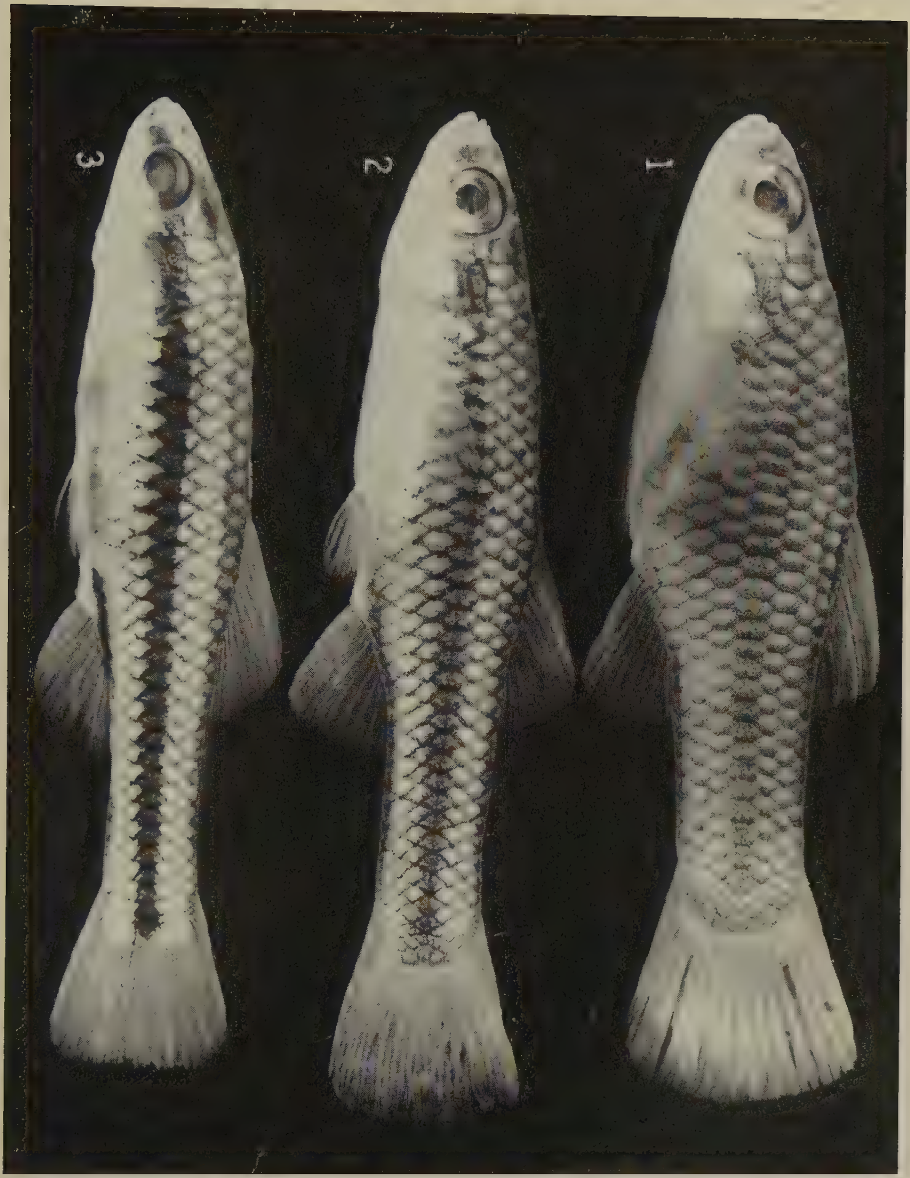
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PLATE VI

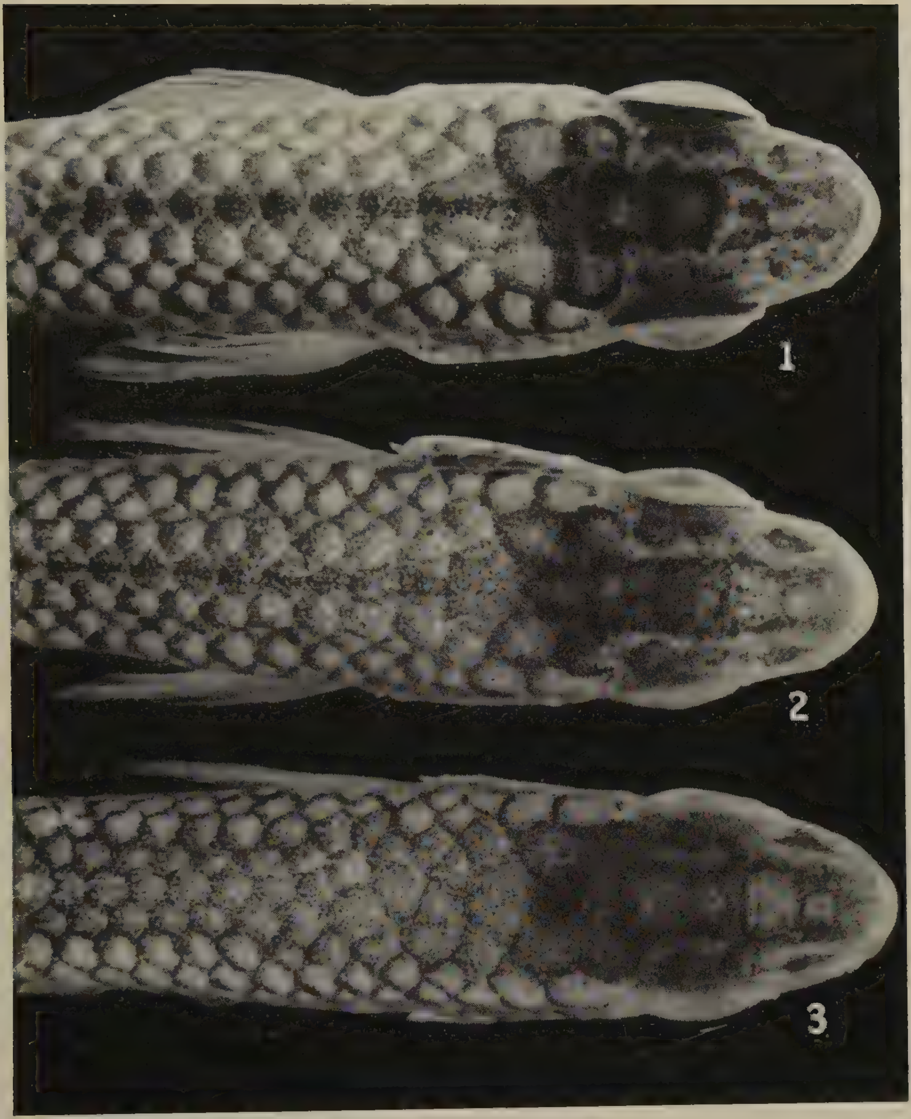
Dorsal views of head and nape of hybrid cyprinodont, *Chriopeops goodei* × *Lucania parva,* and of the parental species

All from St. Marks Migratory Bird Refuge, Florida. Photographed to the same scale by Clarence Flaten.

Fig. 1. *Lucania parva,* male, from collection H41–15.

Fig. 2. Male hybrid, 28.7 mm. in standard length, from same collection.

Fig. 3. *Chriopeops goodei,* male, from same collection.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CONTRIBUTIONS FROM THE LABORATORY OF VERTEBRATE BIOLOGY

UNIVERSITY OF MICHIGAN, ANN ARBOR, MICHIGAN

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BIOLOGICAL AND MORPHOLOGICAL DISTINCTNESS OF A PREVIOUSLY UNDESCRIBED SPECIES OF THE *PEROMYSCUS TRUEI* GROUP FROM TEXAS

BY W. FRANK BLAIR

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Introduction 1
Isolation of the Texas Population 2
Fertility Relations 2
Morphological Distinctness of the Texas Mice 3
Evolutionary Status of the Texas Mice 6
Peromyscus comanche, New Species 7
Summary 8
Literature Cited 8

INTRODUCTION

STOCKS of mice that superficially resemble the brush mouse (*Peromyscus boylii*) were collected in 1932 and 1933 by M. F. Landwer from Tule Canyon, Briscoe County, and from Cooper's Canyon, Garza County, Texas. Subsequent breeding tests and the study of skeletal characters at the Laboratory of Vertebrate Biology show that the affinities of these mice lie not with the *boylii* group but with the *truei* group.

Because the Tule Canyon stock was maintained in the laboratory for ten years, it is possible to take into account certain biological as well as strictly morphological data in determining the relationships of this population to already known and described mice of the *truei* group. The Cooper's Canyon stock was not developed as a laboratory stock, and only four skulls and one skin of field-caught individuals are available.

I am indebted to M. F. Landwer for permission to describe this new form, and to W. H. Burt, of the University of Michigan Museum of Zoology, for making available certain field-killed specimens. I am indebted to H. H. T. Jackson, of the United States Fish and Wildlife Service, for the loan of topotypes of *Peromyscus nasutus* from the collection under his care. The matings of the laboratory mice were made under the direction of L. R. Dice, who also made the body measurements. The cytological preparations and determinations were made by Ray Moree, of the University of Michigan Department of Zoology. Skeletal measurements were made by S. L. Bellanca and by myself. Statistical calculations were made by D. M. Clarke, Bruce Kirchenbaum, and myself.

ISOLATION OF THE TEXAS POPULATION

The population of mice of which the Tule Canyon and Cooper's Canyon stocks are samples probably occupies only a restricted range on the rocky, cedar-covered slopes along the "Break of the Plains" in western Texas. This population lives more than one hundred miles from the nearest other known population of mice of the *truei* group. The nearest known specimens of *nasutus* have been recorded from Tucumcari, Quay County, New Mexico (Bailey, 1931: 157), and *truei* and *nasutus* have been taken in the extreme northwestern corner of the Oklahoma Panhandle (Blair, 1939: 122).

In New Mexico, both *truei* and *nasutus* are mostly restricted to the sabinal and montane life belts of the mountain slopes, where they are most abundant in the pinyon-juniper association (Dice, 1942: 205). In New Mexico, both *truei* and *nasutus* range short distances on the desert grasslands, but nowhere have they been found more than two or three miles from the base of the mountains (Blair, 1941: 226). Therefore, the more than one hundred miles of treeless, relatively level, relatively rockless, short-grass plains lying between the Texas population and the nearest populations of *truei* and *nasutus* must be an effective barrier.

FERTILITY RELATIONS

The Tule Canyon mice can be crossed in the laboratory with *nasutus* from Arizona and New Mexico, although not without some difficulty. Five of twenty matings between Tule Canyon mice and *nasutus* were successful, and resulted in the production of seven male and two female hybrids. The percentage of successful matings in this cross was somewhat smaller than in the pure stocks. Among thirty-one matings within the stock of *nasutus* from Lincoln, New Mexico, only twelve (39 per cent) were productive. The matings within the Tule Canyon stock were more successful, for fourteen (74 per cent) of nineteen matings produced young. The fact that the cross matings were less successful than the matings within the stocks suggests, but does not necessarily prove, partial infertility between the Tule Canyon mice and *nasutus*.

Six of the F_1 male hybrids were mated with Tule Canyon females, and one of these matings resulted in the birth of one male and one female offspring. Three of the F_1 males were mated unsuccessfully to *nasutus* females. One F_1 female hybrid was mated unsuccessfully with a Tule Canyon male. The fact that only one back-cross mating produced young suggests that there was lowered fertility of the hybrids.

Cytological examination of the testes of the hybrid males affords positive evidence of partial infertility between the Tule Canyon mice and *nasutus*. The testes of four F_1 hybrid males that did not reproduce were prepared and examined by Ray Moree. In one animal, spermatogenesis appeared to be going on more or less normally, and there was an abundance of normal as well as some abnormal sperm. Moree believes that this animal was potentially fertile. The three others examined all showed abnormalities in development. No normal sperm were found in any of them, but a few abnormal young sperm were present in each. Moree classifies these animals as of a sterile type. According to Moree, "the general situation in the Tule Canyon hybrids parallels very closely, if not identically, that found in *truei-nasutus* hybrids." Moree plans to publish a detailed description of the cytological conditions of the hybrids between the Tule Canyon mice and *nasutus.*

According to the evidence the Tule Canyon mice are incompletely fertile with mice of the species *nasutus.* The partial infertility is due, in part at least, to gross disturbances in spermatogenesis. Unfortunately, no attempts were made to cross the Tule Canyon mice with *truei,* which is also partly fertile in crosses with *nasutus.* Nine attempted crosses with mice of the *boylii* group proved unsuccessful.

MORPHOLOGICAL DISTINCTNESS OF THE TEXAS MICE

Laboratory-bred animals of known age provide the only critical means of comparing the Tule Canyon mice morphologically with other mice of the *truei* group. For these comparisons, mice of the one-year age class (36–78 weeks) from the Tule Canyon stock, *nasutus* from Lincoln, Lincoln County, New Mexico, and *truei,* from Capitan Gap, Lincoln County, New Mexico, have been used. Means and standard errors of four body and eleven skeletal measurements have been computed (Table I). The body measurements and the first five skeletal measurements in Table I are standard measurements (Dice, 1932: 5). The others need explanation. The interparietal length is the greatest length, and the interparietal width is the greatest width of that bone. The length of the bulla is its greatest length. The width of the bulla is measured just anterior to the thickened base of the external auditory meatus; this is not the greatest width. The parietal-bullar depth is measured from the median surface of the skull just anterior to the interparietal to the plane of the most inflated parts of the two auditory bullae. The nasal length is the greatest length along the suture between the two nasals. Of bilateral structures, the left member is measured. Means of the same measurements of field-killed Tule Canyon mice and field-killed topotypes of *nasutus* have been included in the table. These field-killed animals are of little use, however, because of their small numbers, unknown ages, and because the body measurements were made by different collectors.

In comparing the two means, the difference between them is considered to be significant if it is 2.7 or more times its standard error.

The Tule Canyon mice are significantly smaller than either *truei* or *nasutus* in a majority of the measurements. They are smaller than *nasutus* in tail length, foot length, femur length, length of mandible, condyle-premaxillary distance, condyle-zygoma distance, length of interparietal, and parietal-bullar depth. They are significantly smaller than *truei* in body

TABLE I

MEASUREMENTS OF *Peromyscus comanche*, *P. nasutus*, AND *P. truei*
Means and standard errors, in millimeters

Stock	No.	Mean Age in Weeks	Parents	Body Length	Tail Length	Foot Length	Ear Length
Laboratory-bred, 1-year class							
nasutus, Lincoln	64	52	7	104.89 ± .37	103.73 ± .61	23.275 ± .064	22.435 ± .096
comanche, Tule Canyon	54	53	6	104.11 ± .44	100.21 ± .59	22.520 ± .097	22.196 ± .122
truei, Capitan Gap	79	54	8	107.53 ± .40	94.17 ± .73	22.977 ± .069	25.196 ± .086
Field-killed							
nasutus, Estes Park	2	......	......	97.50	97.00	23.000	......
comanche, Tule Canyon	10	......	......	88.22	100.11	21.967	......

Stock	Length of Femur	Length of Mandible	Condyle-Premaxilla	Condyle-Zygoma	Bullar Width of Skull
Laboratory-bred, 1-year class					
nasutus, Lincoln	18.234 ± .066	17.562 ± .052	27.266 ± .066	19.374 ± .052	12.206 ± .040
comanche, Tule Canyon	17.900 ± .061	16.898 ± .060	26.620 ± .076	18.962 ± .053	12.124 ± .040
truei, Capitan Gap	19.156 ± .087	17.228 ± .043	27.052 ± .063	19.283 ± .046	12.435 ± .030
Field-killed					
nasutus, Estes Park	......	17.750	26.600	19.000	12.200
comanche, Tule Canyon	......	16.220	25.370	18.330	11.970

Stock	Interparietal Length	Interparietal Width	Length of Bulla	Width of Bulla	Parietal-Bullar Depth	Nasal Length
Laboratory-bred, 1-year class						
nasutus, Lincoln	10.680 ± .061	3.871 ± .038	5.153 ± .023	2.824 ± .024	10.260 ± .030	11.341 ± .040
comanche, Tule Canyon	9.952 ± .082	4.008 ± .164	5.340 ± .028	3.335 ± .025	9.958 ± .030	11.258 ± .165
truei, Capitan Gap	10.343 ± .051	3.608 ± .035	5.720 ± .018	3.424 ± .017	10.271 ± .032	11.223 ± .035
Field-killed						
nasutus, Estes Park	10.700	3.650	4.900	3.050	10.000	10.750
comanche, Tule Canyon	10.000	3.990	5.170	3.140	9.700	10.510

length, foot length, ear length, femur length, length of mandible, condyle-premaxillary distance, condyle-zygoma distance, bullar width of skull, length of interparietal, length of bulla, and parietal-bullar depth.

In only two measurements, length of bulla and width of bulla, are the Tule Canyon mice significantly larger than is *nasutus*. In only one measurement, tail length, are they significantly larger than *truei*.

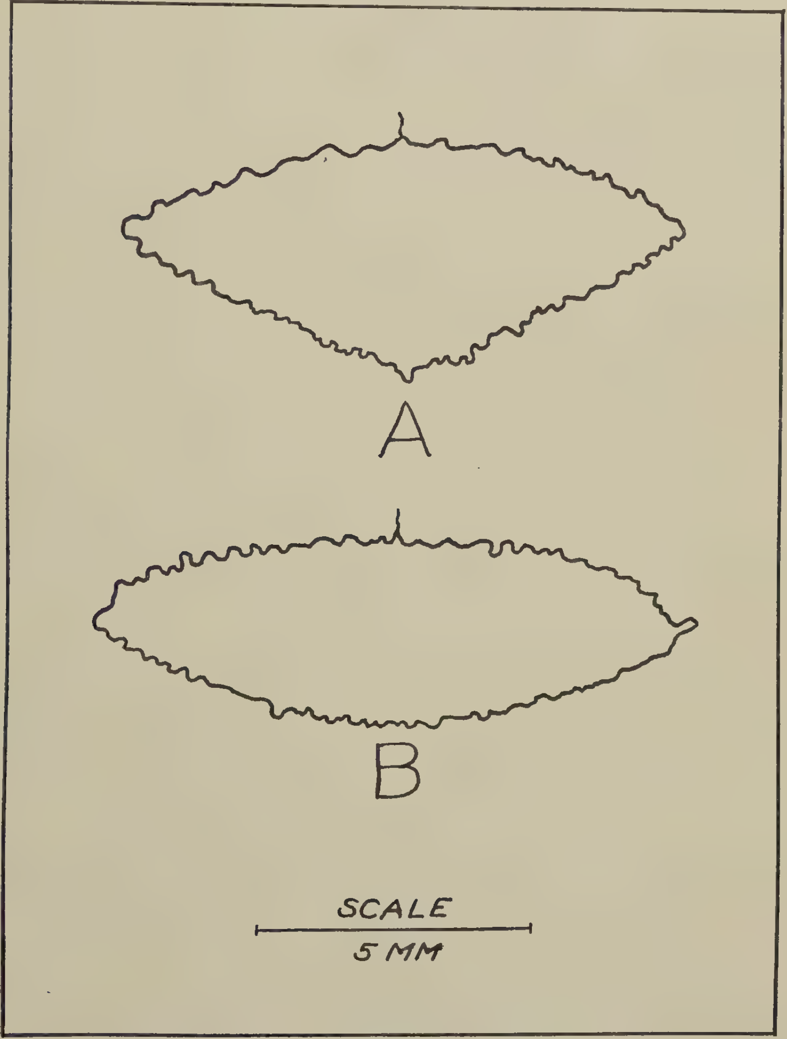

FIG. 1. Interparietal bone of *Peromyscus comanche* (A) aged forty-eight weeks, from the Tule Canyon, Texas, stock and of *Peromyscus nasutus* (B) aged fifty-one weeks, from the Lincoln, New Mexico, stock.

The Tule Canyon mice do not differ significantly from *nasutus* in body length, ear length, bullar width of skull, width of interparietal, or nasal length. They do not differ from *truei* in width of interparietal, width of bulla, or nasal length.

The principal characters by which *truei* and *nasutus* may be separated are the larger ears, larger auditory bullae, and shorter tail of *truei*. By use of these characters mature *truei* and *nasutus* may be readily separated. In all of these characters the Tule Canyon mice are much more like *nasutus* than like *truei,* which shows them to be more closely related to the former than to the latter.

The most conspicuous skeletal difference between *nasutus* and the Tule Canyon mice is in the shape of the interparietal. This bone is roughly elliptical in *nasutus,* but it is triangular in the Tule Canyon mice (Fig. 1). The two species nearly always can be separated by examination of the interparietal, which has much the same shape in *truei* as in the Tule Canyon mice, so that it would be impossible to separate the two on this character alone. The interparietal in *truei* seems to be more variable in shape than in either *nasutus* or in the Tule Canyon mice.

In pelage color the Tule Canyon mice differ from *nasutus* in much the same way that *truei* does. Tule Canyon mice of the one-year class are more brightly buffy than are specimens of *nasutus* of comparable age from Lincoln and from Capitan, New Mexico. Like *truei* but unlike *nasutus,* they have also a lateral stripe of buffy hairs unmixed with gray. Field-killed Tule Canyon mice likewise are more buffy than are field-killed specimens of *nasutus* from Estes Park and Manitou, Colorado, and from Kenton, Oklahoma, and unlike them, have a noticeable lateral stripe.

The Cooper's Canyon mice have slightly smaller auditory bullae than do the mice from Tule Canyon. Otherwise, they are morphologically very much like the Tule Canyon mice.

EVOLUTIONARY STATUS OF THE TEXAS MICE

The three separate breeding arrays that are represented by *nasutus, truei,* and the Texas mice demonstrate two stages in the differentiation of a species from a previously interbreeding population. The populations known as *nasutus* and *truei* have, in some manner and at some time in the past, split into discrete breeding arrays. The divergence of these two populations, presumably through differential mutation pressure and selection pressure, now has reached the point where (1) there is partial infertility between the two; (2) the two kinds occur together in the same ecological associations without interbreeding; and (3) the two are readily separable because of conspicuous morphological differences.

The Texas population and the species *nasutus* are also isolated arrays of a presumably previously interbreeding population. The Tule Canyon mice, like *truei,* have developed partial infertility with *nasutus.* They, too, are prevented from interbreeding with *nasutus,* although in this case by a geographical barrier. They and *nasutus* also have drifted apart morphologically to the point where most individuals of the two kinds may be readily separated on structural characters.

The Texas mice and *nasutus* seem to have been separated a shorter time than have *nasutus* and *truei* for two reasons. First, there is good reason to believe that geographic isolation usually is the first critical step in speciation. After geographic isolation has permitted the random divergence of the previously interbreeding populations, the differentiating kinds may again come to occupy the same areas and the same ecological associations without interbreeding. The Texas mice have obviously reached only the stage of geographic isolation, while the *truei* and *nasutus* have passed this stage and have come to occupy in some places common ranges and common ecological associations. Second, the morphological differences between *nasutus* and the Texas mice are not quite so great as those between *nasutus* and *truei*.

The *truei* species group, as now known, is made up of three discrete breeding arrays, no one of which, however, is completely infertile with the others. This species group corresponds to my concept of a cenospecies (see Blair, in press), for the three breeding arrays are at least partly fertile *inter se* but infertile with other cenospecies. In classification, the Texas population of mice must rank as a taxonomic species, just as do *truei* and *nasutus*. This species may be described as follows.

Peromyscus comanche, new species

The Texas Juniper-mouse

TYPE.—Female adult, skull and skin; Univ. Mich. Mus. Zool. No. 66901; from Tule Canyon, Briscoe County, Texas, about 22 miles east of Tulia; May 23, 1932; collected by M. F. Landwer; original number, 237.

GEOGRAPHIC DISTRIBUTION.—Known only from the type locality and from Cooper's Canyon, four miles southwest of Post, Garza County. Probably occurs in other canyons along the Break of the Plains in northwestern Texas.

CHARACTERS.—Ears and bullae much smaller than in *truei;* tail longer than in *truei.* Interparietal triangular rather than elliptical as in *nasutus.* Pelage brighter than in *nasutus,* with a pronounced lateral line of buffy hairs unmixed with gray. Skull larger and braincase more inflated than in *boylii,* with much larger auditory bullae.

MEASUREMENTS OF TYPE (in mm.).—Total length, 206; tail vertebrae, 107; hind foot, 22.0; ear from notch, 21.0. Skeleton: greatest length of skull, 28.5; basilar length of Hensel, 21.0; zygomatic breadth, 14.0; interorbital constriction, 4.5; length of interparietal, 9.7; width of interparietal, 3.8; length of nasals, 11.0; shelf of bony palate, 4.1; palatine slits, 5.5; diastema, 7.0; postpalatal length, 10.1; maxillary tooth row, 4.2; condyle-premaxilla distance, 26.7; condyle-zygoma distance, 19.0; bullar width of skull, 12.3; length of bulla, 5.4; width of bulla, 3.3; parietal-bullar depth, 10.0.

COLOR (worn pelage refers to type; unworn to laboratory raised animals).—Ground color of dorsal pelage Ochraceous Buff[1] mixed with dusky, fading into Light Ochraceous Buff in worn pelage. There is a pronounced lateral line of Ochraceous Buff unmixed with dusky. General effect above of Cinnamon or Tawny Olive in unworn pelage, changing to Wood Brown in worn pelage. Sides of face and nose grayish. Ears dusky. Tail more or less sharply bicolor. Feet and underparts white.

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[1] Capitalized color terms are after Ridgway (1912).

SPECIMENS EXAMINED.—*Peromyscus comanche:* Tule Canyon, Briscoe Co., Texas, 92; Cooper's Canyon, Garza Co., Texas, 4. *P. n. nasutus:* Lincoln, Lincoln Co., New Mexico, 65; Capitan, Lincoln Co., New Mexico, 13; Estes Park, Larimer Co., Colorado, 4; Manitou, El Paso Co., Colorado, 5; Taft Canyon, Cimarron Co., Oklahoma, 1. *P. t. truei:* Capitan Gap, Lincoln Co., New Mexico, 80. *P. boylii attwateri:* New Braunfels, Comal Co., Texas, 4. *P. boylii rowleyi:* Summerhaven, Pima Co., Arizona, 14.

SUMMARY

A population of mice of the *Peromyscus truei* group living in Tule Canyon and adjacent canyons along the Break of the Plains in Texas is geographically isolated from other mice of the same species group. The Tule Canyon mice are only partly fertile in crosses with specimens of *nasutus* of the *truei* group. Morphologically, they are more like *nasutus* than *truei*, but they differ considerably from *nasutus* both in skeletal structure and in external appearance. The Tule Canyon mice are here described as a new species, *Peromyscus comanche*, of the *truei* group.

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CONTRIBUTIONS FROM THE LABORATORY OF VERTEBRATE BIOLOGY

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INHERITANCE OF THE WHITE–CHEEK CHARACTER IN MICE OF THE GENUS *PEROMYSCUS*

BY W. FRANK BLAIR

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INTRODUCTION

IN beach mice of the races *Peromyscus polionotus leucocephalus* and *P. p. albifrons* pigmented hairs are restricted to the dorsal body surface. The hairs of the ventral and lateral surfaces are white throughout. In some mice of these races, the area of unpigmented hairs extends somewhat on the dorsal surface. Mice of the race *leucocephalus* have the least extensive area of dorsal, pigmented hairs. These mice have fully pigmented hairs only along the middorsal line. The hairs bordering the middorsal stripe are pigmented only at the base. All other body hairs are pure white. This coat pattern may be called the *leucocephalus* type.

The coat pattern of the *leucocephalus* and *albifrons* mice contrasts strongly with the pigmentation pattern of mice of the race *P. p. polionotus* and of all members of the related species *maniculatus*. The species *polionotus* and *maniculatus* are both members of the *maniculatus* species group. In the coat pattern usual for the mice of the *maniculatus* group, the hairs of the dorsal surface are pigmented throughout; and the ventral and lateral hairs, except a few in the inguinal region and on the chin, are pigmented at the base and only the tips are white. This pattern of pigmentation may be called the *maniculatus* type.

An analysis of the genetic differences between the *leucocephalus* and *maniculatus* patterns of pigmentation was made by Sumner (1930). He showed that the *leucocephalus* pattern is partly dominant over the *manicu-*

latus type and that the total difference between the two types is due to the effects of several genes. Sumner, however, did not isolate any of these factors. The present report deals with the mode of inheritance of one of these pattern genes.

It is especially desirable to analyze the genetic differences between the two types of coat pattern of these mice, because these patterns of pigmentation are adaptive in nature. The race *leucocephalus* lives on Santa Rosa Island, which is a white sand reef. The mice of this race are to our eyes relatively inconspicuous on the white sand, because their lateral surfaces and part of their dorsal surface is white. A mouse with the *maniculatus* pattern of pigmentation would on the contrary be easily seen on the white sand. Mice of the race *albifrons,* which are more extensively pigmented above than are those of *leucocephalus,* are likewise well adapted for concealment on the somewhat darker mainland beaches on which they live. Mice of the race *polionotus,* which have the *maniculatus* pattern, are inconspicuous on the dark soils that they inhabit in the interior of Georgia and Alabama. On the other hand, a mouse with the *leucocephalus* pattern would be highly conspicuous on these dark soils.

The chi-square test has been used to determine the significance of deviations from the expected ratios in the F_2 and backcross generations. With one degree of freedom a deviation is considered significant if the chi-square value exceeds 3.841, which marks the 5 per cent level of probability. A deviation is considered highly significant if the chi-square exceeds 6.635, which marks the 1 per cent level of probability.

I am indebted to Don Hayne for permission to examine several stocks of *Peromyscus polionotus* that he collected in western Florida. I am indebted to Elizabeth Barto for suggestions concerning the test for linkage with another set of factors and for critically reading the manuscript.

THE WHITE-CHEEK CHARACTER

The F_1 Generation

Cross matings first were made between mice of the *maniculatus* type, as represented by a stock of *Peromyscus maniculatus bairdii* from southwestern Michigan, and a strain of the *leucocephalus* type that had been selected for minimum extent of pigmented hairs. This *leucocephalus* strain was obtained by selection following a cross of *leucocephalus* from Santa Rosa Island with *albifrons* from the mainland of Florida. Crosses were later made between *Peromyscus maniculatus blandus* from New Mexico and *leucocephalus* from Santa Rosa Island.

The F_1 hybrids that were obtained from these crosses varied considerably in the distribution of pigmented hairs, but all of the sixty-three animals had one conspicuous character in common. The fur hairs on the cheeks up to the lower rim of the eyes were white in at least their distal band (Pl. I). In some individuals, moreover, the fur hairs in this region were white throughout their length. At least some of the guard hairs in this region, however, were dark at their tips. The white-cheek effect obtained results from the extension of the white pigmentation pattern that is characteristic of the ventral surfaces of the head and body upward toward the dorsal head surface. This character I propose to call white cheek (*Wc*) in contradistinction to the usual "colored cheek" (*wc*) of mice that have the *maniculatus* pattern of pigmentation. In the colored-cheek mice the lower rim of the eye is bordered by hairs that are pigmented to their tips.

On the hypothesis that the white cheek represents the effect of a major pattern gene that is inherited as a simple dominant over the colored cheek, the hybrids were backcrossed to colored-cheek mice, and an F_2 generation also was raised.

The F_2 Generation

Thirty-one F_2 hybrids of the cross between *bairdii* and the selected *leucocephalus*-type strain were raised. As only three F_1 males were produced in this cross, and as two of these proved sterile, all of the F_2 hybrids were the offspring of a single pair of mice. Twenty-one white-cheeked and ten colored-cheeked mice were obtained in the F_2 generation. The deviation from the expected 3 : 1 ratio is not significant, for the chi-square value is only 0.871, with one degree of freedom. The segregation in this generation agrees with the hypothesis that white cheek is a single unit dominant over colored cheek.

The Backcross to Colored Cheek

The most convincing proof that white cheek is a single unit dominant over colored cheek is found in the segregation in the backcross generation, for a much larger number of individuals was produced in this cross than in the F_2. The backcross to *bairdii* of the F_1 *bairdii* × *leucocephalus*-type strain produced fifty-eight offspring. The backcross of the F_1 *blandus* × *leucocephalus* hybrids to *blandus* has produced thirty-one offspring. In a testcross to colored-cheeked mice designed to test for linkage between white cheek and the buff phase the progeny totaled 203 individuals. The white-cheek animals used in the testcross were obtained from a second generation of backcrosses to colored-cheek mice. Among the 292 backcross animals in these three groups, 159 were phenotypically white cheek, and 133 were colored cheek. The deviation from the expected 1 : 1 ratio is not significant, for the chi-square value is only 2.315, with one degree of freedom. Therefore, the segregation in both the F_2 and backcross generations indicates that white cheek is a single unit dominant over colored cheek.

TEST FOR LINKAGE WITH BUFF–GRAY

A test was made for linkage between the white-cheek or colored-cheek alleles (*Wc*, *wc*) and the buff-gray factors (A^b, a). This test was made in the coupling relationship. Buff, white-cheeked mice, some or all of them heterozygous for colored cheek, were mated to gray, colored-cheeked mice. Buff, white-cheeked animals ($A^b\ Wc/a\ wc$) obtained from this cross were used in testcross matings, where they were mated to the double recessive, gray, colored-cheek *blandus*. The offspring of the testcross were classified at an age of four to five months.

Buff (A^b) and gray (a) have been shown by Dice (1933) to be alleles, of which buff is dominant. This later was confirmed by Clark (1938). The symbols are those used by Clark, although it has not been proved that buff belongs in the agouti series. The buff used here came from the *bairdii* used in the crosses between *bairdii* and the selected *leucocephalus*-type strain of mice. The white cheek came from the selected strain. The gray and the colored cheek came from *blandus*.

TABLE I

TEST FOR LINKAGE BETWEEN WHITE-CHEEK, COLORED-CHEEK (Wc, wc), AND BUFF-GRAY (A^b, a)

Testcross

($A^b\ Wc/a\ wc \times a\ wc/a\ wc$)

Class	*Observed*	*Expected*
Buff, white cheek	63	50.75
Gray, white cheek	49	50.75
Buff, colored cheek	46	50.75
Gray, colored cheek	45	50.75
Total	203	203.00

ANALYSIS OF CHI-SQUARE

Source of Variation	*Degrees of Freedom*	*Chi-Square*
White cheek: colored cheek ratio	1	2.172
Buff: gray ratio	1	1.108
Linkage	1	0.833
Total	3	4.113

The testcross generation comprised 203 animals. Of these, sixty-three were buff, white cheek; forty-nine were gray, white cheek; forty-six were buff, colored cheek; and forty-five were gray, colored cheek (Table I). The buff white-cheek and the gray colored-cheek types are the parental classes. The gray white-cheek and the buff colored-cheek classes are recombinations. There is no significant deviation from the simple 1:1 ratio between recombinations and parental types that would be expected in the absence of any linkage between the genes Wc and A^b. The chi-square value is only 0.833, which is not significant. At the 1 per cent level of probability, however, the numbers are not sufficient to disprove a possible linkage having more than 37.8 per cent crossing over.

The results of this test indicate, therefore, that the genes Wc and A^b either are on different chromosomes or are very loosely linked.

GENETIC BASIS FOR THE *leucocephalus* PATTERN

The gene *Wc* accounts for only a part of the difference in coat pattern between the coast and island races of the species *polionotus* and the other members of the *maniculatus* species group. The total extension of white in these races is due to the interaction of several genes, as Sumner (1930) has already pointed out. An investigation of these other pattern genes is now in progress, and only a general statement about them is possible at this time.

White-cheek segregants in F_2 and backcross generations vary considerably in the degree of extension of white hairs over other parts of the body. Five grades of reduction of pigmented area of *Wc* mice can be recognized: (1) unmodified white cheek, which presumably represents the effect of only the gene *Wc*, (2) tail white, in which the dorsal hairs of the tail are white to their bases, (3) femoral white, in which the hairs on the inside of the thigh, in addition to the dorsal tail hairs, are pure white, (4) belly white, in which the abdominal and thoracic regions as well as the dorsal tail surface and inner thigh have white hairs, and (5) side white, in which the white area extends up onto the sides of the body. The wild type of *leucocephalus* represents at least one additional stage in reduction of the pigmented area, but this grade has not yet been obtained in the hybrids.

The highest grade of reduction of the pigmented area (side white) can be obtained from the lower grades (tail white, femoral white, belly white) by selective breeding. Conversely, the extent of the pigmented area is increased by successive backcrossing to *maniculatus*-type mice, which, because of their distant geographic origin, presumably carry few if any of the minor genes for the extension of white. Two or three backcrosses to the *maniculatus*-type animals usually result in the elimination of all completely white hairs except on the cheek and in the regions that are white in the *maniculatus* type.

The colored-cheek segregants in F_2 and backcross generations usually have a pattern of pigmentation identical with that of the parent, colored-cheek stock. Some individuals in these generations, however, have pure white hairs in the femoral region or sometimes in both the femoral and belly regions. These individuals, unlike white-cheek segregants with white femoral regions and bellies, have the dorsal surface of the tail pigmented.

From such evidence as is available, it seems probable that most of the minor pattern genes are expressed only when the gene *Wc* is present. The great variation in the extent of pigmentation of the ventral hairs in the white-cheek segregants compared with the slight variation in the colored-cheek segregants leads to this conclusion. On the other hand, the white thighs and bellies of some colored-cheek segregants indicate that some of the pattern genes reach expression in the absence of *Wc*.

The distribution of all-white hairs over the body in the house mouse is controlled by several spotting factors (see Dunn and Charles, 1937, and Dunn, 1942). My evidence, as well as that obtained by Sumner (1930), suggests that several factors also are involved in producing the *leucocephalus* type of pigmentation.

DISTRIBUTION OF WHITE-CHEEK IN NATURAL POPULATIONS OF *Peromyscus*

The distribution of the gene *Wc* in wild populations of the species *polionotus* is known only in a general way. More than three hundred wild-caught individuals of *leucocephalus* from Santa Rosa Island have been examined without the finding of any colored-cheeked animals. In addition, no colored-cheeked young appeared among the forty-two offspring of crosses between *leucocephalus* and colored-cheeked specimens of *maniculatus*. It seems likely, therefore, that the colored-cheek allele is rare in the Santa Rosa Island population.

Several stocks of the species *polionotus* that were collected by Don Hayne in western Florida are in the collection of the Laboratory. Most of the specimens available are laboratory bred, so they give no precise measure of the gene frequencies in the wild populations. However, they and such field-caught animals as are available indicate that inland from approximately the line between western Florida and Alabama the mice are colored cheeked. In the strip approximately forty miles wide between the Gulf coast and the Alabama line, both white-cheeked and colored-cheeked mice occur. On some mainland beaches the mice appear to be nearly all white cheeked; on others the populations are mixed.

The populations of the nearly white coastal beaches may be thought of as a great reservoir of white-cheek genes and of the minor color pattern genes as well. The populations of the inland, dark soils of Alabama may likewise be considered a reservoir of colored-cheek genes. Western Florida, between the coast and approximately the Alabama line is a tension zone into which are dispersed the remarkably different genotypes of the populations on each side of it. Detailed studies of the gene frequencies in the populations of this tension zone, when they can be made, will be of the highest importance to an understanding of the dynamics of raciation.

SUMMARY

The white-cheek character (*Wc*) of *Peromyscus polionotus leucocephalus* and *P. p. albifrons* is a single-unit dominant over the colored cheek (*wc*) of the species *Peromyscus maniculatus*. The genes *Wc, wc,* and those for buff and gray, A^b, *a,* either lie on different chromosomes or are very loosely linked.

The differences in pattern of pigmentation between the *polionotus*-type mice of the Florida coast and the inland mice of *maniculatus* type are produced by the gene *Wc* acting together with several other genes. These other genes, as yet unanalyzed, eliminate pigment from the hairs on various parts of the body.

The mice of the white beaches and islands of western Florida are mostly white cheeked, and in addition they usually carry other genes that reduce the extent of pigmented hairs on the body. The mice of the dark, inland soils of Alabama are colored cheeked. In western Florida, both white-cheeked and colored-cheeked mice occur intermingled over a strip extending approximately forty miles inland from the Gulf of Mexico.

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PLATE I

Fig. 1. White-cheek pattern of mouse derived from crosses between *Peromyscus polionotus* and *P. maniculatus.*

Fig. 2. Gray-cheek pattern of deer-mouse of *Peromyscus maniculatus blandus* stock from Alamogordo, New Mexico.

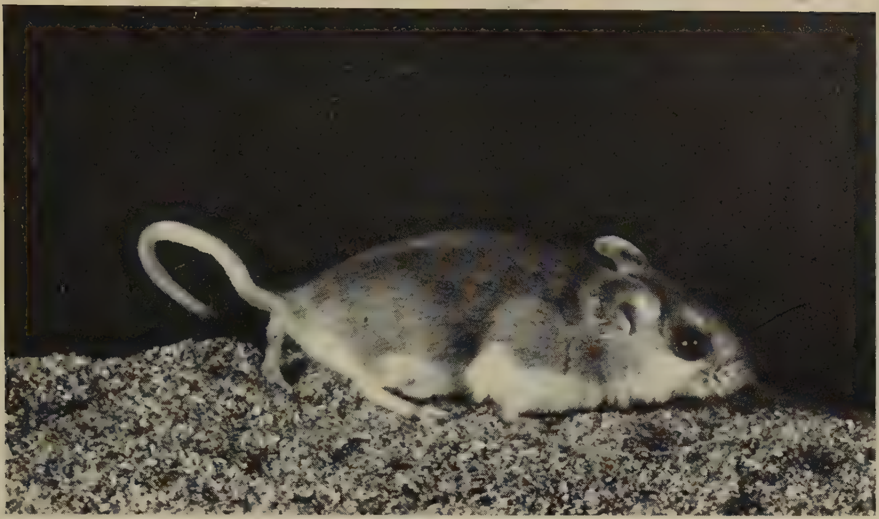

FIG. 1

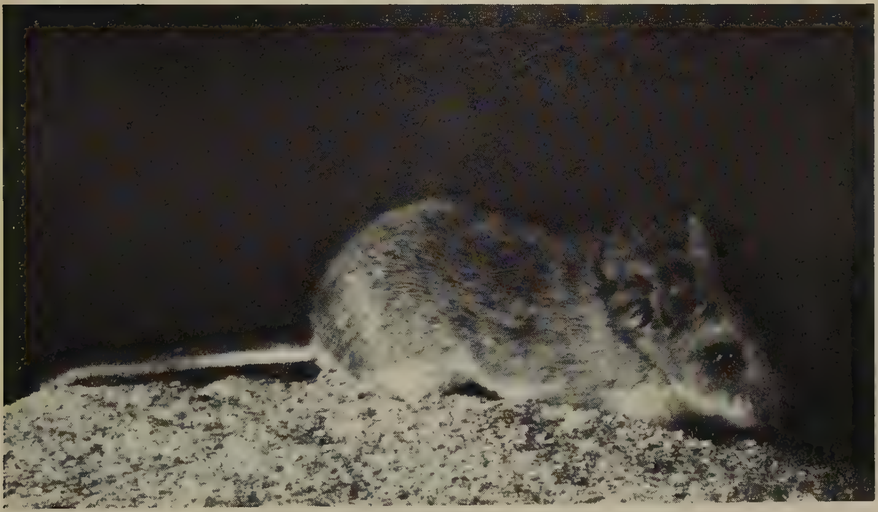

FIG. 2

CONTRIBUTIONS FROM THE LABORATORY OF VERTEBRATE BIOLOGY

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EXPERIMENTAL EVIDENCE OF SEXUAL ISOLATION BETWEEN THREE FORMS OF MICE OF THE CENOSPECIES *PEROMYSCUS MANICULATUS*

BY W. FRANK BLAIR AND WALTER E. HOWARD

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INTRODUCTION

THIS report deals with an experimental test for sexual, or psychological, isolation between three forms of the *Peromyscus maniculatus* species group. A method is described by which the sexual isolation and social behavior of mammals may be studied experimentally. The term sexual or psychological isolation is used here in the sense that it is used by Dobzhansky (1941: 257). It is assumed that copulation between individuals of sexually isolated species fails to occur because of the lack of mutual attraction.

Sexual isolation is a highly important factor in speciation. Once sexual isolation has been achieved between two geographically isolated populations it acts as a barrier to interbreeding that probably can be surmounted only with difficulty if the two populations again come to have overlapping ranges. The stage at which sexual isolation develops is an important one in the differentiation of biological kinds. Some biologists (Dobzhansky, 1937 and 1941; Mayr, 1940 and 1942; and others) believe that the attainment of sexual isolation is the most important event in the differentiation of a species. W. F. Blair (1943) believes that the acquirement of sexual isolation is second only to the development of intersterility as an important stage in differentiation.

No previous attempt to study experimentally the degree of sexual isolation of different kinds of wild mammals is known to us. Dice (1940 and 1942) reasons that some sort of psychological isolating mechanism must operate to prevent the interbreeding of mammals that in the laboratory will hybridize and produce at least some fertile offspring, but which in the field maintain discrete breeding arrays where their ranges overlap.

Sexual isolation has been demonstrated experimentally in *Drosophila* (Sturtevant, 1920; Dobzhansky and Koller, 1938; and Stalker, 1942) and in *Bufo* (A. P. Blair, 1942). Our methods are similar to those of previous workers in that a choice of mating partners is offered the captive animals. The relatively complex social behavior of mammals, however, requires a special technique. With such highly organized forms, the social behavior as well as actual copulation is indicative of the presence or absence of psychological isolating mechanisms.

The experiments were planned by us jointly, and the daily records were made by one or both of us. The report has been written by the senior author, as the junior author was called into military service before the experiments were completed. We are indebted to Charles W. Cotterman for suggestions concerning the statistical treatment of the data and to Carl L. Hubbs for critically reading the manuscript. In the absence of both authors in army service the manuscript has been supervised through the press by Lee R. Dice.

METHODS

Our method of investigation consisted simply of creating an artificial society in a group of laboratory cages. Within this society each mouse could associate with an individual of the opposite sex of its own kind or with either of a pair of mice of a different kind kept in the same group of cages.

Experimental Conditions

CAGES.—Four mouse cages of wire screen, each with floor dimensions of 9 by 16 inches, were connected through the sides by means of short, wooden tunnels. Every mouse could move freely into any cage of the four. Each cage was provided with a food glass, a water bottle, a nest pan, and cotton nesting material. The cages were lettered A to D, to facilitate the keeping of records.

ENVIRONMENTAL CONDITIONS.—The experiments were carried out in a room with northern exposure. No control of temperature was possible during the summer, but during the winter months the room was kept at approximately 72° F.

Breeding condition of the mice.—Only healthy, sexually active mice were tested. It was impossible to use only animals of the same age, but this seemed unnecessary, since all were sexually mature. The breeding condition of these mice can be determined by the condition of the external genitalia. Females going through the estrous cycle have the vagina open, and sexually active males have enlarged testes, located in the scrotum.

Experimental Routine and Treatment of Data

Each experiment was begun by placing four mice, one male and one female of each of the two kinds being tested, in cage A. The mice then were free to distribute themselves through the four cages in any way that suited them. As there were four cages and four nests, each mouse could live in a separate cage and separate nest if it so desired. Each experiment was continued until a litter was born to one of the females, or until a period of approximately forty-five days, which is equal to twice the average gestation period of these mice, had elapsed. Each experimental set was examined daily between eight and ten o'clock in the morning. The mice are active at night and retire to their nests during the day. There was thus obtained a daily record of which mice nested together. As daylight did not come until nearly nine o'clock (Eastern War Time) during the winter months, it was necessary to turn on bright lights in the room for about an hour before all of the mice had retired. When the daily examinations were made the mice were examined for evidence of recent fighting, and the females for possible pregnancy. After an experiment was terminated, the females were segregated and examined frequently until it was evident that they were not pregnant. In order to prevent the carrying over from one experiment to another of any attraction between two individuals, the same pair was never placed together in more than one experiment. Some individuals, however, were reused in a different type of experiment.

The data secured from these experiments are of three types. First, there are the daily records of association in the nests. These may be treated statistically. Second, there are the observations on the fighting that took place in the artificial society made up of two pairs of mice. Third, there is the positive evidence of the choice of mating partners as shown by the paternity of the litters that were born. For most of the litters here reported it is possible to tell the paternity of the young by their appearance. In interpreting the evidence from the production of litters it must be remembered that some of these stocks are only partly fertile with one another, and it is quite possible that pairing and copulation sometimes may take place without the production of offspring. Each of the three kinds of data throws some light on the problem of sexual isolation, and together they afford a measure of the sexual isolation of the mice.

Fifteen different social combinations are possible between the four mice living together in each experiment. These combinations may be grouped according to the type of behavior they indicate. First, and most critical as a test of sexual isolation, are the combinations of one male with one female. Three combinations are possible in which likes (mice of the same type) are paired (see first three columns of social combination scores in Table I). The mice of each kind can be paired simultaneously, or those of either kind can be paired while the other two mice are living singly. We use the word pair to indicate the occurrence together of a male and female of the same stock or strain to the exclusion of the other mice. These three classes of scores will, therefore, be referred to as pair scores. Opposed to the pair scores are the three classes in which a male of one stock or strain has nested with a female of the other type (see last three columns of social combination scores in Table I). These will be referred to as cross-pair scores.

Four combinations are possible in which three mice nest together to the exclusion of the fourth. In referring to these scores, the excluded animal will be spoken of as ''out,'' meaning it is out of the aggregation. Other possible combinations are: (1) males together, females living singly, (2) females together, males living singly, (3) males together in one cage, females together in another, (4) all four mice together, and (5) no two mice together.

In the statistical treatment of the data, the chi-square test of significance has been used. The probability of the occurrence of each social combination on a chance basis is shown at the bottom of each table. In all critical comparisons, however, events of equal probability only are compared, so that a formula for testing a simple 1:1 ratio has been used. There is one degree of freedom. A deviation from the expected equal numbers is considered significant if the chi-square value exceeds 3.841, which marks the 5 per cent level of probability. A deviation is considered highly significant if the chi-square exceeds 6.635, which marks the 1 per cent level of probability.

STOCKS

Origin of the Stocks

Three stocks of mice and the hybrids between them were used in the experiments. The origin of these stocks is as follows:

Leucocephalus, Navarre.—First and second laboratory-born generations from animals that were trapped on Santa Rosa Island, Florida, in the winter and spring of 1942.

Albifrons, Gulf Shores.—Second and third laboratory-born generations from animals trapped on the mainland beaches of Gulf Shores State Park, Alabama, in the winter of 1941–42.

Blandus, Tularosa.—This stock came from the vicinity of Tularosa, Otero County, New Mexico. It has been in the laboratory about four years and has been separated into a number of color strains. Where pairs of *blandus* were used in the experiments only mice of the same color strain were used.

Hybrids.—These were laboratory-produced, F_1 hybrids between *blandus* Tularosa and *leucocephalus* Navarre, and between *blandus* Tularosa and *albifrons* Gulf Shores.

Relationships of the Stocks

The stock of *Peromyscus maniculatus blandus* is representative of the species *maniculatus.* The stocks of *Peromyscus polionotus leucocephalus* and *P. p. albifrons* are representatives of the species *polionotus.* All of these mice belong to the cenospecies *Peromyscus maniculatus* (W. F. Blair, 1943). All will cross in the laboratory if no choice of mating partners is given, but only a small proportion of the species crosses are successful, and in at least the *leucocephalus–blandus* cross some F_1 males are sterile. All of the wild populations represented by these mice are geographically isolated from one another. The population of *leucocephalus* occupies Santa Rosa Island, off the west coast of Florida. The population of *albifrons* occurs on the adjacent mainland and is separated from the nearest representative of the species *maniculatus* by a gap of more than one hundred miles in which neither kind occurs.

THE MATING EXPERIMENTS

Ninety mating experiments were conducted between July, 1942, and February, 1943. In addition to the tests between the different stocks and those between the hybrids and their parent stocks, experiments were carried out in which all mice were from the same stock.

blandus with *blandus*

Sixteen experiments were conducted with *blandus* alone. In these experiments the mice of one pair were of one color strain, whereas those of the other pair were of a different color strain. Four color strains, designated tentatively as dark gray, red, sand, and yellow were used. The sands and yellows differ from the reds and grays in at least one major genetic factor. They are homozygous for a recessive gene for paleness, while most of the reds and dark grays are homozygous for its dominant allele, which produces a dark pelage. The dark and pale mice are conspicuously different in shade. In all of the *blandus* experiments a male and female from one of the pale strains has been tested against a male and a female from one of the dark strains.

The records of the *blandus* with *blandus* tests, except for two that will be discussed later, are summarized in Table I. It is obvious from the summarized daily scores that the distribution of the mice through the four cages was not according to chance. The double-pair and double cross-pair scores and the scores in which the pale mice were paired have a higher frequency than can be attributed to chance. Likewise, the scores in which one or the other male was out of the aggregation and the scores in which all four mice were together exceed the expected frequencies. The frequencies of the scores in which one or the other female was out of the aggregation are very close to the expected. In all of the other classes, the observed frequencies are less than would be expected on the basis of chance.

Dominance among males.—*Blandus* is definitely polygamous, at least in a society such as existed in the experiments. One male usually fights the other male, establishes dominance over him, and associates himself with the two females. The dominant male seems to be complete master over the other male. The losing male usually is bitten severely on the tail, sometimes until the tail is completely lost. The rump is also frequently attacked. The hind feet, scrotum, and ears are bitten occasionally. The dominated male is sometimes killed. The dominant male seldom receives so much as a scratch, for the other male usually submits without attempting retaliation.

In nine of the fourteen experiments listed in Table I, one male showed strong dominance by fighting and defeating the other. In the five remaining experiments, also, one male was dominant, but less strikingly so. Two additional experiments, not listed in Table I, had one dominant male in each. In one of these experiments a male was killed on the first night. In the second experiment one male was so completely cowed that he lived in the food glass, consequently the daily scores from this experiment are not comparable statistically with those from the others. In eleven experiments the pale male was dominant, and in five the dark male.

The male that was dominant in fighting also usually associated with the females to the partial exclusion of the other male. This is shown by comparing the daily scores of the dominant with the dominated male. The dominant male was in a nest with both females while the other male was alone a total of 104 times. Only nine times was the dominated male in a nest with the two females while the other male was alone. This deviation from the 1:1 ratio expected by chance is very highly significant, for the chi-square value is 79.9.

The association of the dominant male with the females also may be shown by lumping all scores in which the dominant male was with one or the other or both of the females while the other male was alone, and comparing the result with the lumped scores in which the dominated male was with one or both of the females. The dominant male was with one or both females 186 times, but the other male was with them only forty-three times. The chi-square value for the deviation from the expected 1:1 ratio is 89.3, which is very highly significant.

The pale male was with one or both females a total of 148 times, and the dark male was with the females only eighty-one times. This deviation from the expected 1:1 ratio is highly significant, for the chi-square value is 19.6. The dominance of the pale male was expressed by his frequent pairing with one or the other of the females, not by association with both of them at the same time. There is no significant difference in the latter type scores of the two males. The pale male was with both females to the exclusion of the dark male a total of sixty-five times, and the dark male was with both females forty-eight times. The chi-square value for the deviation from the expected 1:1 ratio is only 2.6, which is not significant.

TABLE I

Social Groups Formed in an Experimental Society of *Peromyscus blandus* with *blandus*

One pair of pale strain (P) together with one pair of dark strain (D) in each experiment.

Experiment Number	Total Daily Records	Social Combinations															Litters			Fighting	Analysis
		Double Pairs	Dark ♂ with Dark ♀	Pale ♂ with Pale ♀	Dark ♀ Out	Pale ♀ Out	Pale ♂ Out	Dark ♂ Out	None Together	♂♂ Together	♀♀ Together	All 4 Together	♂♂ Together ♀♀ Together	Pale ♂ with Dark ♀	Dark ♂ with Pale ♀	Double Cross Pairs	Likes × Likes	Cross Matings	Father?		
31	39	......	1	......	......	......	2	16	......	......	7	......	5	7	......	1		D × P	P × ?	♂ D often bitten ♂ P bitten once	One ♂ dominant
39	37	1	1	16	......	......	......	8	5	2	1	......	......	3	......	......			P × ?	♂ D often bitten	One ♂ dominant
53	22	2	......	2	......	2	......	11	1	......	2	......	......	2	......	......	P × P (2)			♂ D often bitten	One ♂ dominant
61	27	3	7	1	2	2	5	......	......	......	3	2	......	......	2	......	D × D (2)	P × D		♂ P often bitten ♀ D stole yg. of ♀ P	One ♂ dominant
85	52	1	6	3	......	......	26	2	......	1	4	3	......	1	1	4		P × D		♂ P often bitten	One ♂ dominant
86	25	2	1	......	3	2	3	7	......	......	1	3	2	......	1	......		D × P		♂ D often bitten	One ♂ dominant
54	29	......	......	3	1	1	1	8	1	......	1	1	......	4	2	6	P × P	P × D		♂ D often bitten	♂ D mated with ♀ P, then ♂ P dominated
76	48	2	......	......	4	1	11	1	......	......	2	18	1	......	2	6				♂ P bitten once	All together, then ♂ D dominant
60	27	7	......	6	......	......	......	6	1	......	1	......	......	5	......	1		P × D		One ♂ D killed 2d ♂ D often bitten	♂ P dominant, but ♂ D parent of only litter
56	31	2	......	1	6	6	......	2	......	4	1	3	1	......	......	5	P × P (2)	D × P		♂ D chased once by a P	♂ P sired litters. ♀ ♀ pregnant and often alone
73	26	......	......	18	1	......	......	......	2	1	......	......	......	......	4	......	D × D (2)			♂ P often bitten	♂ and ♀ P paired ♂ D dominant
84	29	9	1	4	3	......	......	2	1	1	......	5	......	......	1	2		D × P (2)		♀ D often bitten ♂ D bitten once	Often paired ♂ P dominant
65	30	11	2	5	1	......	......	2	......	......	1	......	......	2	1	5		D × P		♀ P and ♂ D bitten	Definite pairing and cross pairing
71	24	1	......	......	2	5	......	......	1	2	1	9	1	......	......	2		P × D		♀ D bitten twice ♂ P killed after litter born	Together, or pregnant ♀ P out
Total	446	41	19	59	23	19	48	65	12	11	25	44	10	24	14	32	9	11	2	Frequent fighting	One ♂ dominant
Expected	446	20.9	41.8	41.8	20.9	20.9	20.9	20.9	41.8	41.8	41.8	7.0	20.9	41.8	41.8	20.9					

The dominant males were the fathers of most of the litters conceived during the experiments. Nineteen litters were fathered by dominant males, and only two were sired by nondominant males. This deviation from the expected 1:1 ratio is highly significant, for the chi-square value is 13.8. In both cases in which nondominant males fathered litters the pregnancies took place near the beginning of the experiments and possibly before the dominance of the other male was established. In one of these cases the same female later produced a litter by the dominant male.

Not all of the fighting was restricted to the males. In three experiments one female was bitten a few times, but we do not know which individual did the biting. An interesting item of female behavior was observed in one experiment (No. 61) in which both females bore litters at the same time. The dark female stole the young of the pale female. These young also were dark as they were fathered by a dark male. This female moved the stolen young into her nest and nursed them along with her own offspring. When the experiment was later broken up the stolen young were left with their foster mother, who raised them as well as her own to maturity.

Assortative mating of color types.—A considerable amount of pairing occurred in the *blandus* experiments, even though one male usually dominated the other. To determine whether or not a nesting partner of the same color strain is selected, the three classes of pair scores may be lumped and compared directly with the sum of the three classes of cross-pair scores.

The total frequency of the pair scores is 119, and that of the cross-pair scores is only seventy. Since the probabilities of the occurrence of either pairing or cross pairing are equal, the chi-square test may be used to test the significance of the deviation from the expected, simple 1:1 ratio. The chi-square value is 12.7, which indicates a highly significant deviation in the direction of the assortative pairing by color strains.

The pale mice were paired more frequently (fifty-nine times) than were the dark mice (nineteen times). Double-pair scores are not included here, because the two types of pairs obviously cancel one another. To include them would unjustifiably multiply the data. The chi-square value for testing the deviation from the expected 1:1 ratio is 20.5, which indicates a highly significant deviation in the direction of pairing of pale with pale.

In actual breeding, as shown by the litters produced, there was no selection by color type by the parents. Twenty-three litters were born. Two died or were eaten before their paternity could be determined. Of the other twenty-one, nine were the result of matings between mice of the same color strain, and twelve were the result of cross matings between strains. There is no significant deviation from an expected 1:1 ratio, for the chi-square value for the deviation is only 0.4.

leucocephalus with *leucocephalus*

Seven experiments were carried out with *leucocephalus* alone. Two males and two females were placed together just as in all the other experiments, but here there were no known genetic differences between any of the mice.

The social behavior of *leucocephalus* is very different from that of *blandus*. There was no evidence of fighting between any of the *leucocephalus* individuals, and all four mice in an experiment usually lived together in the same nest (see Table II). In only one experiment (No. 93) was there any obvious departure from this behavior. In this experiment, the two males frequently nested with the pregnant female while the nonpregnant female was alone. There possibly was some antagonism between the two females.

The least probable combination on the basis of chance (all four mice together) had a frequency greater than the total frequency of all other combinations. In 190 daily records (70.4 per cent of the total) all four of the mice were living together. The observed frequencies of the combinations in which only three mice were together were close to the expected frequencies of those combinations. Also, the observed frequency of the combination in which the males were together in one cage, the females in another, was close to the expected. The frequencies of all other combinations were much below the expected. There was no significant amount of pairing between any two individuals.

Even the birth of a litter did not cause any fighting among the *leucocephalus* individuals. In one experiment (No. 38) both females produced young before they were removed from the experimental cages. After the birth of the second litter, five days after the advent of the first, the two females moved their respective offspring into the same nest. There the two litters became mixed, and each female occasionally nursed young of both litters.

Another habit of undoubted social significance is the storing of food by *leucocephalus*. Sunflower seeds were dropped into the cages once weekly. The mice carefully stored every sunflower seed in one or two of the unused nest pans. The same storage place was used throughout each experiment. If the nest pan was lifted, and the seeds scattered about, they were carefully placed back in the nest pan the following night. No food was stored by *blandus*.

albifrons with *albifrons*

Six experiments were conducted with *albifrons* alone. The results are shown in Table II. The behavior of *albifrons* bore some resemblance to that of *leucocephalus*, but there were obvious differences. There was a tendency for all of the animals in an experiment to nest together, but in only 29.5 per cent of the daily records were all four together, compared with 70.4 per cent for the *leucocephalus*.

Definite pairs were formed and maintained in one experiment (No. 57). This is the only experiment in all of our series in which actual mating behavior was observed. The morning after a litter was born to female A, this female and male A were observed running through the cages. Both mice

TABLE II

Social Groups Formed in an Experimental Society of *Peromyscus*

Two males (♂A and ♂B) and two females (♀A and ♀B) of the same form together in each experiment.

Experiment Number	Total Daily Records	Social Combinations															Litters	Fighting	Analysis
		Double Pairs	♂A with ♀A	♂B with ♀B	♀B Out	♀A Out	♂A Out	♂B Out	None Together	♂♂ Together	♀♀ Together	All Together	♂♀ Together ♂♀ Together	♂A with ♀B	♂B with ♀A	Double Cross Pairs			
leucocephalus with *leucocephalus*																			
34	45	1	......	......	......	3	......	1	......	......	......	36	2	......	......	2	....	No evidence of fighting; seeds stored	Lived together amicably
38	31	......	......	......	1	......	......	2	......	......	......	28	......	......	......	......	♀A × ❢ ♀B × ❢	No evidence of fighting; seeds stored	Lived together amicably
42	43	......	......	......	3	1	2	3	......	......	1	30	1	1	......	1	....	No evidence of fighting; seeds stored	Lived together amicably
47	48	2	......	......	......	1	2	5	......	......	1	36	1	......	......	......	....	No evidence of fighting; seeds stored	Lived together amicably
67	35	......	......	......	2	1	3	3	......	1	......	25	......	......	......	......	♀B × ❢(2)	No evidence of fighting; seeds stored	Lived together amicably
92	39	......	......	......	4	2	2	1	......	......	......	27	3	......	......	......	♀B × ❢(2)	No evidence of fighting; seeds stored	Lived together amicably
93	29	......	......	......	11	......	6	......	......	1	......	8	2	......	1	......	♀A × ❢	No evidence of fighting; seeds stored	All together or 2 ♂♂ with pregnant ♀A
Total	270	3	......	......	21	8	15	15	......	2	2	190	9	1	1	3	7	No fighting	Strongly gregarious
Expected	270	12.6	25.3	25.3	12.6	12.6	12.6	12.6	25.3	25.3	25.3	4.2	12.6	25.3	25.3	12.6			
albifrons with *albifrons*																			
57	46	14	13	7	1	5	1	......	......	......	2	......	......	2	1	......	♀A × ❢	♀B bitten once	Definite pairing
51	36	2	2	2	4	3	......	3	1	......	......	14	......	1	1	3	♀A × ❢(2)	No fighting, stored seeds	Together or temporary pairing
64	29	1	......	1	3	2	1	2	......	......	......	16	1	......	1	1	♀A × ❢	No fighting evident	Lived together amicably
98	31	......	......	......	3	2	1	4	......	1	2	15	3	......	......	......	....		
117	26	1	1	......	6	2	......	4	......	......	......	7	1	2	......	2	♀B × ❢	♂B often bitten	♂A dominant
121	25	2	1	......	......	......	10	3	......	......	1	5	1	......	......	2	♀A × ❢	No fighting evident	♂B dominant
Total	193	20	17	10	17	14	13	16	1	1	5	57	6	5	3	8	6	Some fighting	Moderately gregarious
Expected	193	9.0	18.1	18.1	9.0	9.0	9.0	9.0	18.1	18.1	18.1	3.0	9.0	18.1	18.1	9.0			

were obviously excited, and the male made several attempts to mount the female. The other pair of mice in the experiment were somewhat excited too, but the other male made no attempt to copulate with this female.

One male dominated the other in two experiments (Nos. 117 and 121). In the first of these one male was badly bitten, and in the second, one male was with the females more frequently than was the other. Fighting occurred in two of the six experiments. In addition to the experiment in which a male was badly bitten, there was one in which a female was bitten once. Seeds were stored in only one experiment, and then they were stored only for a short period.

The social behavior of *albifrons* resembles that of *blandus* in the tendency to pair and in the fact that one male sometimes dominates the other. In frequently nesting together the *albifrons* resemble *leucocephalus*. Individuals of *albifrons* are less inclined to store food than are those of *leucocephalus*, and they fight more than do *leucocephalus*, but less than *blandus*. In general, therefore, the behavior of *albifrons* is somewhat intermediate between that of *leucocephalus* and that of *blandus*.

leucocephalus with *blandus*

Seventeen experiments were set up with one pair of *leucocephalus* and one pair of *blandus* (Table III). In each set the male and female of *blandus* were of the same color strain. In eleven of the experiments there was definite pairing by kinds to the exclusion of most other combinations. In two of the others (Nos. 78 and 100) the two males associated with the female *leucocephalus* after the female *blandus* became pregnant. In the four remaining experiments, all four mice were together more frequently than they were in any other combination. The six experiments in which there was little pairing are scarcely less indicative of species discrimination than the experiments in which there was definite pairing. The fact that the males were nesting together a majority of the time in these experiments indicates to us that the psychological differences between the two kinds are so great that little feeling of competition exists between the males.

The frequency of occurrence of double-pair and *leucocephalus*-pair scores is much greater than would be expected on the basis of chance. The frequency of the *blandus*-pair scores is close to the expected frequency. The three classes of cross-pair scores all fall far below the frequencies expected by chance. The combinations of all four mice together and of three together with the *blandus* female out exceed the expected frequencies. The scores of the remaining combinations are close to or less than the expected frequencies.

There was at least a little fighting in eleven of the experiments. The fighting was very different, however, from that in the *blandus* controls, where one male was continually bullied by the other. Here the fighting was sporadic, and one individual was seldom bitten more than once. Females were involved in fighting as much as males; eight individuals of each sex showed evidence of injury from fighting. The fighting was about evenly divided between the forms, for six of the *blandus* and ten of the *leucocephalus* showed evidences of injury. The one exception to the general rule of little fighting occurred in experiment No. 59, in which both *leucocephalus* were killed by the *blandus*.

The sexual isolation between *leucocephalus* and *blandus* is best shown by a comparison of the combined pair scores with the combined cross-pair scores. The pair scores total 394, and the cross-pair scores total only twenty-one. This is a very highly significant deviation from the 1:1 ratio that would be expected in the absence of any discrimination between the two kinds, for the chi-square value is 335.3.

The *blandus* male was with the two females while the other male was alone more frequently than was the *leucocephalus* male. The *blandus* male was with the two females thirty-one times, and the *leucocephalus* male was with them only fifteen times. The chi-square value for testing the deviation from the expected 1:1 ratio is 5.6, which is significant. This probably indicates slight dominance by the *blandus* male. That this dominance, if it is such, is negligible is shown by the fact that the *blandus* male entered into cross pairs no more frequently than did the *leucocephalus* male.

All of the litters born in the experiment that survived until identifications could be made were the result of the mating of likes. Two litters born to *blandus* were killed soon after birth. Nine litters of *blandus* and two litters of *leucocephalus* were identified. Thus, there is no evidence of cross mating under the conditions of the experiment. We cannot rule out the possibility of copulation not followed by pregnancies between mice of the different kinds, because the two kinds are only partly fertile together.

albifrons with *blandus*

Seventeen experiments were conducted with *albifrons* combined with *blandus*. The results are given in Table IV. In five experiments, likes were paired most of the time. In seven experiments, all four of the mice nested together more frequently than they occurred together in any other combination. In two experiments (Nos. 74 and 89) the *blandus* male was dominant. In one experiment (No. 72) the mice were either all four together or cross paired most of the time. In the two remaining experiments (Nos. 83 and 113) the female *blandus* usually was alone, while the two males lived with the female *albifrons*. In one of these the *blandus* female was pregnant, and in the other the female *blandus* possibly bore a litter that was eaten before we found it. Fighting occurred in eleven experiments, the same number as in the *leucocephalus* with *blandus* tests, and in general the fighting was of the same magnitude as in those tests.

Four combinations occurred much more frequently than would be expected on the basis of chance. These were (1) double pairs, (2) all four mice together, (3) *blandus* female out of the aggregation, and (4) *albifrons*

TABLE III

SOCIAL GROUPS FORMED IN AN EXPERIMENTAL SOCIETY OF *PEROMYSCUS*

One pair of *leucocephalus* (L) together with one pair of *blandus* (B) in each experiment.

Experiment Number	Total Daily Records	Social Combinations																Litters			Fighting	Analysis
		Double Pairs	*blandus* ♂, ♀ Together	*leucocephalus* ♂, ♀ Together	*blandus* ♀ Out	*leucocephalus* ♀ Out	*leucocephalus* ♂ Out	*blandus* ♂ Out	None Together	♂♂ Together	♀♀ Together	All Together	♀♀ Together ♂♂ Together	*leucocephalus* ♂ with *blandus*	*blandus* ♂ with *leucocephalus* ♀	Double Cross Pairs	B × B	L × L	Father ?		
115	26	13	5	3	......	1	......	1	2	1	......	......	......	......	......	......	2			No fighting evident	Definite pairing
33	34	23	4	......	1	......	3	......	......	......	......	......	......	......	3	......			♀B × ?		
79	44	28	8	8	......	......	......	......	......	......	......	......	......	......	......	......				♀ B bitten once	Definite pairing
37	43	7	3	13	2	3	......	1	3	3	......	......	......	6	......	2				No fighting evident	Definite pairing
62	40	32	6	......	......	1	......	......	......	......	......	1	......	......	......	......	2			Both B often bitten	Definite pairing
20	48	18	11	11	1	......	2	......	1	......	1	......	......	1	2	......				♂L wild. ♀L once chased ♀B	Definite pairing
22	42	22	2	16	......	......	......	1	1	......	......	......	......	......	......	......				♂ B, ♀ L bitten once	Definite pairing
23	52	25	12	7	3	......	......	......	......	1	1	......	......	......	3	......	1			♀ L bitten once	Definite pairing
32	42	25	2	15	......	......	......	......	......	......	......	......	......	......	......	......	1			Both L bitten once	Definite pairing
35	45	19	1	18	3	1	......	......	......	2	......	......	......	......	1	......				Two ♀♀ fighting once	Definite pairing
59	6	2	......	2	......	......	2	......	......	......	......	......	......	......	......	......				Both L killed	Definite pairing
78	26	1	......	......	15	2	......	1	......	......	......	6	1	......	......	......			♀B × ?	No fighting evident	After ♀ B pregnant both ♂♂ usually with ♀ L
100	29	2	2	3	13	4	......	......	......	1	1	1	2	......	......	......	1			♀ L bitten once	
87	22	1	......	2	1	1	8	2	......	......	......	7	......	......	......	......	1	1		♀ L bitten once	All together or ♂ L out
29	44	3	......	5	8	5	2	4	......	......	......	15	......	1	......	1				No fighting evident	All usually together or one out
116	26	4	1	1	......	......	8	4	......	......	......	8	......	......	......	......	1	1		No fighting evident	All usually together or one ♂ out
118	30	7	1	......	......	1	6	1	......	......	......	13	......	......	......	1				♀ L bitten once. ♂ B chased ♀ L once	All usually together or paired
Total	599	232	58	104	47	19	31	15	7	8	3	51	3	8	9	4	9	2	2	Occasional fighting	Usually paired
Expected	599	28.1	56.2	56.2	28.1	28.1	28.1	28.1	56.2	56.2	56.2	9.4	28.1	56.2	56.2	28.1					

male out of the aggregation. Three combinations occurred about as frequently as would be expected on a chance basis. These were (1) double cross pairs, (2) *albifrons* female out of the aggregation, and (3) *blandus* male out of the aggregation. All of the other possible combinations occurred much less frequently than would be expected by chance.

Pairing occurred more frequently than did cross pairing. There were 139 instances of pairing compared with sixty-three of cross pairing. The deviation from the 1:1 ratio that would be expected in the absence of species discrimination is highly significant. The chi-square value is 28.6; however, the sexual isolation between *albifrons* and *blandus* is not nearly so great as that between *leucocephalus* and *blandus*. In the tests between *albifrons* and *blandus* 31.1 per cent of all pair and cross-pair scores were cross pairs, and in the tests between *leucocephalus* and *blandus* only 5.1 per cent were cross pairs.

Additional evidence of isolation between these two forms is found in the two experiments in which the two males associated with one female and in the seven experiments in which all four usually nested together. As in the *leucocephalus* with *blandus* test, we believe that the frequent occurrence together of the *albifrons* and *blandus* males indicated that there was relatively little competition between them for mates.

There was considerably more competition between *albifrons* and *blandus* males than there was between *leucocephalus* and *blandus* males. Evidence of competition between males was obvious in two experiments (Nos. 74 and 89). In one, the male *blandus* associated with the females to the exclusion of the *albifrons* male, and in the other the *albifrons* male was killed. Additional evidence that the male *blandus* tended to dominate over the male *albifrons* is in the total daily scores showing association of males and females. The *blandus* male was with both females and the *albifrons* male was alone forty-seven times. The *albifrons* male was with both females and the *blandus* male was alone only twenty times. The chi-square for this deviation from the 1:1 ratio that would be expected in the absence of male dominance is 10.8, which is highly significant.

The *blandus* male was with one or both females while the *albifrons* male was alone a total of 106 times. The *albifrons* male was with one or both females a total of only fifty-one times. The chi-square value for this deviation from the expected 1:1 ratio is 19.3, which is highly significant. These scores indicate that *blandus* males dominate *albifrons* males to a considerable degree under the conditions of the experiment.

All litters that lived long enough to be identified were the result of the mating of like with like. Twenty-one litters were born. Three litters born to *blandus* females and six born to *albifrons* females were killed soon after birth. Eleven litters resulted from the mating of *blandus* with *blandus*, and one was produced by the mating of *albifrons* with *albifrons*. Therefore, all of our evidence indicates that the mice mated only with their own kind. As in the *leucocephalus* with *blandus* test, we cannot be certain that unsuccessful copulation between individuals of the two different forms did not occur.

blandus–leucocephalus Hybrids with *blandus*

Ten experiments were conducted in which F_1 hybrids between *blandus* and *leucocephalus* were tested with *blandus* (Table V). In two of the experiments (Nos. 90 and 107) F_1 *leucocephalus* × *blandus* hybrids were used. In the eight others the reciprocal hybrids were used. The two groups are here treated jointly. In only two experiments (Nos. 63 and 70) was there definite pairing of likes. The hybrid male dominated the *blandus* male in seven experiments, and the *blandus* male was dominant in one experiment (No. 69).

Three combinations were considerably more frequent than could be expected on the basis of chance. These were (1) double pairs, (2) male *blandus* out of the aggregation, and (3) all four mice together. Four combinations were considerably less frequent than could be expected on the basis of chance. These were (1) no mice together, (2) males together, (3) females together, and (4) males together in one cage, females together in another. The scores of the other combinations were fairly close to the expected frequencies.

A comparison of the pair and cross-pair scores shows that there was no isolation between the hybrids and the pure *blandus*. Pairs occurred a total of seventy times, and cross pairs sixty times. This deviation from the expected 1:1 ratio is not significant, for the chi-square value is only 0.8. With the production of an F_1 hybrid generation the psychological barrier to the transfer of *leucocephalus* characters to *blandus* is broken down.

Additional evidence of the breakdown of the previously strong sexual isolation is found in the behavior of the males. One male usually was strongly dominant over the other. Just as in the *blandus* controls the dominant male bullied the other and monopolized the company of the females. The hybrid male was strongly dominant in seven experiments, and the *blandus* male was dominant in only one. This dominance by the hybrid males probably is accounted for by the fact that these hybrids are much more vigorous and aggressive than are the pure *blandus*. The dominance of the hybrid males is proved statistically by the scores showing association with the females. The hybrid male nested with both females while the *blandus* male was alone a total of fifty-seven times. The *blandus* male was with both females only seventeen times. The chi-square value for this deviation from the 1:1 ratio that would be expected in the absence of male dominance is 21.6, which is highly significant.

The hybrid male nested with one or both females a total of ninety-eight times while the other male was alone. The *blandus* male nested with one or both females only fifty-four times. The chi-square value for this deviation from the expected 1:1 ratio is 12.7, which is highly significant. Therefore,

TABLE IV

SOCIAL GROUPS FORMED IN AN EXPERIMENTAL SOCIETY OF *PEROMYSCUS*

One pair of *albifrons* (A) together with one pair of *blandus* (B) in each experiment.

Experiment Number	Total Daily Records	Social Combinations Double Pairs	*blandus* ♂, ♀ Together	*albifrons* ♂, ♀ Together	*blandus* ♀ Out	*albifrons* ♀ Out	*albifrons* ♂ Out	*blandus* ♂ Out	None Together	♂♂ Together	♀♀ Together	All Together	♀♀ Together ♂♂ Together	*albifrons* ♂ with *blandus* ♀	*blandus* ♂ with *albifrons* ♀	Double Cross Pairs	Litters B × B	A × A	Father ?	Fighting	Analysis
77	26	11	3	......	2	1	......	2	......	2	......	......	......	......	5	......	1		♀A × ?	♀B often bitten ♀A bitten once	Definite pairing
103	28	12	1	10	4	......	......	......	......	......	......	......	......	......	......	1	1			No fighting evident	Definite pairing
112	31	16	5	2	1	......	4	......	......	......	......	1	......	......	2	......	1	1	♀A × ?	♂ and ♀B fighting once	Definite pairing
120	25	17	1	2	......	1	1	......	......	......	1	2	......	......	......	......			♀A × ? ♀B × ?	All bitten	Definite pairing
119	28	11	1	1	1	1	......	......	1	1	......	7	......	1	2	1	1		♀A × ?	♂B bitten once	Definite pairing
72	22	1	1	......	6	2	2	......	......	......	......	5	......	......	......	5			♀A × ?	No fighting evident	More cross pairing than pairing
74	32	1	2	......	......	......	25	......	......	......	......	......	......	......	3	1	2			♂A bitten once	♂B dominant
89	16	1	......	......	1	......	2	1	1	1	1	1	2	......	5	......				♂A killed	♂B dominant
114	18	......	......	......	......	......	2	......	......	......	1	7	2	1	......	5	1			♂ and ♀A bitten once	Lived together or cross paired
80	30	1	2	1	4	2	4	2	......	......	......	7	5	......	1	1	2			No fighting evident	All frequently together
75	31	1	3	1	1	1	1	3	......	......	......	20	......	......	......	......			♀A × ?	No fighting evident	All frequently together
94	54	3	3	1	4	4	......	6	1	......	......	27	1	......	1	3				No fighting evident	All frequently together
95	26	3	1	......	1	2	2	......	......	1	......	14	......	......	1	1	1			No fighting evident	All frequently together
88	61	......	1	......	11	8	1	......	......	1	1	36	......	......	......	2				♀B bitten once	All frequently together
91	22	1	......	1	......	4	......	4	......	......	......	8	......	1	1	2			♀B × ?	♀B dead last day	All frequently together
113	25	3	1	2	13	......	......	......	......	......	......	......	......	......	4	2	1		♀B × ?	♂ and ♀A often bitten ♀B bitten once	Pregnant ♀B usually alone
83	65	4	1	6	20	3	3	2	1	3	......	11	......	1	8	2				♂A bitten once	♀B alone or all mice often together
Total	540	86	26	27	69	29	47	20	4	9	4	146	10	4	33	26	11	1	9	Moderate fighting	Usually paired or all together
Expected	540	25.3	50.6	50.6	25.3	25.3	25.3	25.3	50.6	50.6	50.6	8.4	25.3	50.6	50.6	25.3					

both the indiscriminate pairing and the presence of strong male dominance indicate the absence of sexual isolation between *blandus* and the *blandus–leucocephalus* hybrids.

The parentage of the litters that were produced offers additional evidence of the breakdown of sexual isolation between the *blandus* and the hybrids. Unfortunately, the paternity of the litters could not always be positively determined, because the hybrids were heterozygous for the definitive characters. Three litters, however, positively were the result of matings between *blandus* females and hybrid males. Four litters definitely were produced by matings between males and females of *blandus,* and three were the result of matings between hybrids. Three litters born to *blandus* females and three born to hybrid females were unidentifiable or were lost. The three litters from matings of *blandus* females and hybrid males afford positive evidence of a breakdown of sexual isolation sufficient to allow interbreeding even when there is a choice of mates.

blandus–leucocephalus Hybrids with *leucocephalus*

Nine tests were made between *leucocephalus* and the F_1 hybrids between *leucocephalus* and *blandus* (Table VI). In one experiment (No. 48) F_1 *leucocephalus* × *blandus* hybrids were used. Reciprocal hybrids were used in the others. All are treated jointly. In two experiments, likes were paired together more often than the mice were associated in any other combination. In four experiments, the *leucocephalus* male dominated the hybrid male. In two experiments, all four of the mice were usually together, and in the remaining experiment the two males were usually with the *leucocephalus* female.

Four combinations occurred more frequently than would be expected on the basis of chance. These were (1) double pairs, (2) hybrid female out of the aggregation, (3) hybrid male out of the aggregation, and (4) all four mice together. Four combinations occurred about as frequently as would be expected on the basis of chance. These were (1) *leucocephalus* paired, (2) *leucocephalus* female out of aggregation, (3) *leucocephalus* male out of aggregation, and (4) *leucocephalus* male cross paired with hybrid female. The remaining combinations occurred less frequently than would be expected by chance.

Pair scores occurred much more frequently than did cross pairs. Likes were together a total of 101 times, while cross pairs occurred only thirty-eight times. This deviation from the 1 : 1 ratio that would be expected in the absence of isolation between the hybrids and the mice of the pure stocks is highly significant, for the chi-square value is 28.6. These results indicate that there is some sexual isolation between *leucocephalus* and the F_1 hybrids.

The *leucocephalus* male usually was more or less dominant over the hybrid male. In the four experiments in which this was most obvious the hybrid male was badly bitten. The *leucocephalus* male was with both females and the hybrid male was alone a total of fifty-one times. The hybrid male was with both females only fifteen times. This departure from the expected 1:1 ratio is highly significant, for the chi-square value is 19.6.

The *leucocephalus* male was with one or both females and the hybrid male was alone a total of one hundred times. The hybrid male, on the other hand, was with one or both females only thirty-nine times. The deviation from the expected 1:1 ratio is highly significant, for the chi-square value is 26.8. Our results show definitely that there was antagonism between the males and that the *leucocephalus* associated with the females more often than did the hybrid male.

Nine litters were produced in this group of experiments. Four were the result of matings between *leucocephalus.* One born to a *leucocephalus* female was lost. Two litters resulted from matings between hybrids. One litter born to a hybrid female was lost, and another was unidentifiable. The number of litters is small, but each of the six that could be identified resulted from the mating of like with like.

blandus–albifrons Hybrids with *blandus*

Only three tests were made between *blandus* and F_1 hybrids between *albifrons* and *blandus,* for only a few of these hybrids were available. In one experiment (No. 102) the hybrids used were F_1 *albifrons* × *blandus,* whereas in the other two the reciprocal hybrids were used. The results are given in Table V. In all three experiments the *blandus* male was somewhat dominant. In one the hybrid male was badly injured, and in another it was badly bitten and finally killed.

The data are too few for statistical treatment, but insofar as they go they indicate that the reaction between *blandus* and *albifrons–blandus* hybrids is essentially like the reaction between *blandus* and the *leucocephalus–blandus* hybrids. There were fourteen records of pairing and seven records of cross pairing. The *blandus* male was dominant, whereas in the test between *blandus* and the *leucocephalus–blandus* hybrids the hybrid male usually dominated. The *blandus* male was with both females and the other male was alone seventeen times, but the hybrid male was with both females only five times. The former male was with one or both females twenty-five times, and the latter was with one or both females only eight times.

One litter, from a mating between *blandus,* was identifiable. Three other litters born to female *blandus* and two born to hybrid females were unidentifiable.

The few data suggest virtual elimination of sexual isolation between *blandus* and the F_1 hybrids, just as in the *leucocephalus–blandus* hybrids.

blandus–albifrons Hybrids with *albifrons*

The results of three tests of *blandus–albifrons* hybrids with *albifrons* are given in Table VI. In one experiment (No. 50) F_1 *albifrons* × *blandus* were

TABLE V

SOCIAL GROUPS FORMED IN AN EXPERIMENTAL SOCIETY OF *PEROMYSCUS*

One pair of *blandus* (B) together with one pair of hybrids (F_1) in each experiment.

Experiment Number	Total Daily Records	Social Combinations															Litters				Fighting	Analysis
		Double Pairs	Hybrid ♂ with Hybrid ♀	*blandus* ♂ with *blandus* ♀	Hybrid ♀ Out	*blandus* ♀ Out	*blandus* ♂ Out	Hybrid ♂ Out	None Together	♂♂ Together	♀♀ Together	All Together	♂♂ Together ♀♀ Together	*blandus* ♂ with Hybrid ♀	Hybrid ♂ with *blandus* ♀	Double Cross Pairs	B × B	$F_1 \times F_1$	B × F_1	Father ?		
blandus with *blandus–leucocephalus* hybrids																						
70	25	12	3	8	......	......	......	......	1	......	1	......	......	......	......	......	1				Both F_1's bitten	Definite pairing
106	24	12	3	......	......	2	......	......	......	......	......	......	......	7	......	......			2		Both F_1's bitten	Definite pairing
63	28	1	2	......	1	......	9	2	......	......	3	2	1	1	2	4				B × ? F_1 × ?	♂B often bitten	F_1♂ dominant
90	26	......	2	......	......	......	10	......	2	......	4	......	......	1	......	3				F_1 × ?	♂B often bitten	F_1♂ dominant
107	30	3	4	......	......	1	13	......	1	......	......	3	2	1	1	1		1		B × ?	♂B often bitten	F_1♂ dominant
82	18	......	......	......	......	......	6	......	1	?	1	......	......	......	7	2				B × ?	♂B often bitten	F_1♂ dominant
105	33	3	2	1	1	......	6	2	3	1	......	1	......	......	7	6		1	1		Both F_1's bitten once Both B often bitten F_1♂ seldom bitten	F_1♂ dominant
97	28	......	......	......	2	......	8	4	......	......	1	9	......	1	......	3		1			No fighting evident	F_1♂ dominant
110	26	3	1	......	4	4	5	......	1	2	......	4	......	......	......	2	1			F_1 × ?	F_1♀ often bitten	F_1♂ dominant
69	30	......	......	10	......	......	......	9	2	......	2	......	......	7	......	......	2				F_1♂ often bitten	♂B dominant
Total	268	34	17	19	8	7	57	17	11	4	12	19	3	18	24	18	4	3	3	6	Frequent fighting	One ♂ usually dominant
Expected	268	12.6	25.1	25.1	12.6	12.6	12.6	12.6	25.1	25.1	25.1	4.2	12.6	25.1	25.1	12.6						
blandus with *blandus–albifrons* hybrids																						
102	20	2	......	4	1	2	3	4	......	......	......	......	2	......	......	2	1				F_1♂ often bitten, finally killed	Pairing and ♂B dominant
111	28	2	1	1	6	4	1	4	......	1	......	7	1	......	......	......				B × ?	F_1♀ often bitten	All together or one out
81	29	1	1	2	1	1	1	9	......	......	1	5	2	1	1	3				B × ? (2) F_1 × ? (2)	F_1♂ often bitten	♂B dominant
Total	77	5	2	7	8	7	5	17	......	1	1	12	5	1	1	5	1	...	...	5	Frequent fighting	One ♂ usually dominant
Expected	77	3.6	7.2	7.2	3.6	3.6	3.6	3.6	7.2	7.2	7.2	1.2	3.6	7.2	7.2	3.6						

used; in the others reciprocals were used. There was definite pairing of likes in one experiment. In the others, all four mice nested together more frequently than they occurred in any other combination.

The data are too few for statistical treatment, but they suggest that the reaction between *albifrons* and the F_1 *blandus–albifrons* hybrids is not greatly different from that between *leucocephalus* and *blandus–leucocephalus* hybrids. Likes were paired twenty-four times, while cross pairs occurred only ten times. The hybrid male associated with the females somewhat more frequently than did the *albifrons* male. The hybrid male was with both females and the *albifrons* male was alone seventeen times, but the *albifrons* male was with the females only eight times. Likewise, the hybrid male was with one or both females twenty-five times, and the *albifrons* male was with one or both only eleven times.

Two litters were born as the result of matings between individuals of *albifrons*. One other litter, born to an *albifrons* female, was lost before it could be identified.

The results suggest that sexual isolation between the hybrids and *albifrons* is greater than that between them and *blandus*.

DISCUSSION

It is shown above that the three forms of mice of the cenospecies *Peromyscus maniculatus* tested differ in their social behavior in an experimental society comprising only two pairs of mice. In a wholly *blandus* society, one male usually fights the other male and establishes dominance over him. The dominant male often associates with the two females to the exclusion of the other male, and he sires most of the young that are produced. The social behavior of *leucocephalus* mice in the experimental society is very different from that of *blandus*. The *leucocephalus* mice are highly social and all four of them usually nested together. These mice are extremely tolerant of one another even when young are in the nest. *Leucocephalus* mice also differ from *blandus* mice in their habit of making community food stores. The behavior of *albifrons* individuals in the experimental society is intermediate between that of *leucocephalus* and that of *blandus*. The *albifrons* mice resemble *leucocephalus* mice in their habit of frequently all nesting together. They also make food stores, but they make them less frequently than do the *leucocephalus* mice. The *albifrons* individuals resemble those of *blandus* in their tendency to form pairs and in the occasional dominance of one male over the other.

Strong sexual isolation between individuals of *leucocephalus* and *blandus* is exhibited in a mixed society made up of these two forms. The two *blandus* usually live by themselves in one nest, and the two *leucocephalus* in a different nest. Only rarely does a male of one stock nest with a female of the other stock. Our evidence indicates that the two kinds of mice do not interbreed under the conditions of the experiment, for all of the litters produced resulted from the mating of *blandus* with *blandus* or of *leucocephalus* with *leucocephalus.* The *blandus* male forms an aggregation with the two females to the exclusion of the other male slightly more often than does the *leucocephalus* male. This possibly indicates slight dominance by the *blandus* male. The deviation, however, from the 1: 1 ratio between the two types of aggregations is not highly significant. Occasional fighting occurs in this society, but, unlike the fighting in the pure *blandus* society, it is sporadic; an individual is seldom bitten more than once during an experiment.

Sexual isolation is strong also between *albifrons* and *blandus,* but it is not nearly so strong as that between *leucocephalus* and *blandus.* This difference in the degree of sexual isolation by which the two forms of *Peromyscus polionotus* are separated from *blandus* is shown by the higher percentage of cross-pair scores in the *blandus–albifrons* society and by the fact that the *blandus* male sometimes dominates the *albifrons* male. In the *blandus–albifrons* society 31.1 per cent of all pair and cross-pair scores were cross pairs, whereas in the *blandus–leucocephalus* society only 5.1 per cent were cross pairs. The *blandus* male associated with the females significantly more frequently than did the *albifrons* male. All of the young that lived long enough to be classified resulted from the mating of *blandus* with *blandus* or of *albifrons* with *albifrons.* The F_1 hybrids between *blandus* and *leucocephalus* and between *blandus* and *albifrons* showed no sexual isolation from pure *blandus.* Cross pairs between *blandus* and the hybrids occurred about as often as did pairs between pure *blandus* or between the hybrids. One male dominated the other as strongly as in the pure *blandus* society. The hybrid male usually dominated the *blandus* male, probably because of its greater vigor and aggressiveness. The dominant male fought the other male and monopolized the company of the females. The hybrids and the pure *blandus* interbred even though the *blandus* individuals had the opportunity of breeding with their own kind.

The sexual isolation between the F_1 hybrids and the *leucocephalus* or *albifrons* stock is less strong than that between the two parent stocks. The extent of isolation between the hybrids and the parent *leucocephalus* or *albifrons* stocks is much greater than that between the hybrids and *blandus.* The mice pair with their own kind more frequently than they cross pair. The *leucocephalus* male usually dominates the hybrid male. This suggests that the differences in the factors affecting sexual isolation between the hybrids and the parent *leucocephalus* are sufficiently small so that the *leucocephalus* male recognizes the hybrid male as a competitor for the females. We are unable to explain why *leucocephalus* males established dominance over hybrid males but not over other *leucocephalus* males. We have obtained no evidence of cross breeding between *leucocephalus* and the hybrids or between *albifrons* and the hybrids under the conditions of the experiment.

Our information about the social behavior of these mice in an unmixed society suggests that differences in social habits are important factors in

TABLE VI

SOCIAL GROUPS FORMED IN AN EXPERIMENTAL SOCIETY OF *PEROMYSCUS*

One pair of a parent stock together with one pair of hybrids in each experiment.

Experiment Number	Total Daily Records	Social Combinations																Litters			Fighting	Analysis
		Double Pairs	Hybrid ♂ with Hybrid ♀	Pure ♂ with Pure ♀	Hybrid ♀ Out	Pure ♀ Out	Pure ♂ Out	Hybrid ♂ Out	None Together	♂♂ Together	♀♀ Together	All Together	♂♂ Together ♀♀ Together	Pure ♂ with Hybrid ♀	Hybrid ♂ with Pure ♀	Double Cross Pairs	Pure × Pure	$F_1 \times F_1$	Father ?		
leucocephalus with *leucocephalus–blandus* hybrids																						
99	32	17	6	......	......	......	1	......	......	......	......	7	......	......	1	......		1		No fighting evident	Definite pairing
48	29	9	1	3	8	2	1	1	......	......	......	......	1	......	2	1			♀L × ?	Both L nearly killed	Definite pairing
101	46	12	2	......	7	......	2	2	......	......	......	20	......	1	......	......				No fighting evident	Together or paired
55	45	5	......	8	16	2	......	4	......	1	2	2	......	3	2	......				No fighting evident	Paired or 2 ♂♂ with ♀L
45	42	3	2	3	5	2	5	4	......	2	......	11	......	2	1	2	1		♀F_1 × ?	No fighting evident	Lived together
43	10	......	......	......	......	......	......	8	......	......	......	......	......	2	......	......				♂ hybrid often bitten	♂L dominant
66	42	7	4	3	......	2	3	7	......	......	2	8	1	5	......	......	2			♂ hybrid often bitten	♂L dominant
104	35	......	1	2	2	2	3	11	......	1	1	1	1	5	2	3		1	♀F_1 × ?	♂ hybrid often bitten	♂L dominant
108	35	6	......	7	......	......	......	14	2	......	......	......	......	5	......	1	1			♂ hybrid often bitten	♂L dominant
Total	316	59	16	26	38	10	15	51	2	4	5	49	3	23	8	7	4	2	3	Some fighting	Paired or ♂L dominant
Expected	316	14.8	29.6	29.6	14.8	14.8	14.8	14.8	29.6	29.6	29.6	4.9	14.8	29.6	29.6	14.8					
albifrons with *albifrons–blandus* hybrids																						
50	32	3	2	1	1	4	6	1	......	......	1	9	......	1	......	3	2			No fighting evident	Lived amicably together
58	34	......	......	......	1	......	6	5	......	......	2	13	2	......	2	3			♀A × ?	No fighting evident	Lived amicably together
109	40	14	3	1	6	2	5	2	......	1	2	3	......	......	1	......				Each ♂ bitten once	Definite pairing
Total	106	17	5	2	8	6	17	8	......	1	5	25	2	1	3	6	2		1	Little fighting	Usually paired or all together.
Expected	106	4.7	9.9	9.9	4.7	4.7	4.7	4.7	9.9	9.9	9.9	1.7	4.7	9.9	9.9	4.7					

sexual isolation. Each of the three stocks tested varies markedly in its social behavior. Significantly, the *albifrons* mice, which differ least from *blandus* mice in social habits, are less effectively isolated from *blandus* than are *leucocephalus* mice, which differ the most.

We do not know the actual psychological factors that cause each *leucocephalus, albifrons,* and *blandus* mouse to associate with and interbreed with another of its own stock rather than with an individual of a different but related form. Many factors probably influence the selection of a mate. Physical characters and behavior patterns as well as more elemental stimuli, some perhaps olfactory in nature, probably play a part in the choice of a mating partner. Visual discrimination of physical characters probably is important in the selection of mates. Such discrimination is suggested by the fact that individual *blandus* of the same color strain paired together more frequently than did those of different colors. It is possible, of course, that the mates were selected for characters associated with the pelage color rather than for the color itself.

The sexual isolation between *leucocephalus* and *blandus* and between *albifrons* and *blandus* is not operative in nature, because both *leucocephalus* and *albifrons* are geographically isolated from any representative of the species *maniculatus.* The sexual isolation, however, that exists here would be of the highest evolutionary importance if, as is very possible, either of these forms came to occupy in the future a range overlapping that of *maniculatus.* In that contingency mating preferences would tend to maintain the distinctness of the populations of the two species.

That psychological isolating mechanisms may sometimes break down in nature is indicated by the fact that they can be broken down in the laboratory by allowing no choice of mates. The possible results to be expected of a partial breakdown of the sexual isolating mechanisms are shown by the reactions between the F_1 hybrids and their parent stocks. The *leucocephalus–blandus* and *albifrons–blandus* hybrids pair indiscriminately with pure *blandus,* and at least the *leucocephalus–blandus* hybrids interbreed freely with *blandus.* Thus, if hybridization should occur through the partial breakdown of sexual isolation, the rapid transfer of *leucocephalus* or *albifrons* genes into the *maniculatus* population would be hindered only by the infertility of a fraction of the hybrids.

The barrier to the transfer of *maniculatus* genes into *leucocephalus* or *albifrons,* on the contrary, is lowered but not completely removed by hybridization. Cross pairing in the experiments between the hybrids and the mice of the parent stocks is much less frequent than is pairing between individuals of the respective parent stocks or between the hybrids. Furthermore, so far as we have observed, there is no crossbreeding between the hybrids and the mice of the parent stocks. Therefore, the transfer of *maniculatus* genes into the *albifrons* or *leucocephalus* population would proceed more slowly than the reciprocal transfer, and it might not occur at all.

Should overlapping of their geographic ranges ever occur, it is, therefore, theoretically possible that a modification of the *maniculatus* population by the introduction of foreign genes might take place without any modification of the *albifrons* or *leucocephalus* population through the introduction of *maniculatus* genes. Hybridization, in this case, presumably would not result in the immediate swamping of either kind. Instead, under these conditions some modification of one form (*maniculatus*) would theoretically occur without a reciprocal modification of the other.

It is significant that *leucocephalus* and *albifrons* differ in the degree of their sexual isolation from a representative race (*blandus*) of the species *maniculatus.* Both *leucocephalus* and *albifrons* are geographically isolated populations that W. F. Blair (1943) considers to be incipient species of the cenospecies *maniculatus.* A considerable difference between these two incipient species is indicated by the difference in the extent of their sexual isolation from *maniculatus.* These different degrees of sexual isolation presumably represent different stages in the evolution of their isolating mechanisms.

The existence of psychological barriers to the interbreeding of these geographically isolated populations indicates that sexual isolation develops under conditions of geographical isolation. The evidence here is purely circumstantial. We believe that mice of the taxonomic species *maniculatus* and *polionotus* formerly comprised one interbreeding population that was broken into two arrays during some Pleistocene interglacial period (W. F. Blair, 1943). If this is the true history of these forms, the present aversions to crossbreeding between them must have developed since their geographic separation. It is logical to believe that geographically isolated populations will drift apart in the psychological, physiological, and morphological characters that influence the selection of a mate. The development of sexual isolation probably comes as a byproduct of random divergence in the characters of the two populations. This is in agreement with the hypothesis of Stalker (1942: 255) that "genetic isolating mechanisms would be acquired . . . . during evolutionary changes which result in increased differences between sub-groups."

SUMMARY

Three geographically isolated forms of mice—*Peromyscus maniculatus blandus, P. polionotus albifrons,* and *P. polionotus leucocephalus*—are found to differ markedly in their social behavior when each is tested in an experimental society comprising only mice of its own kind. The *blandus* males are polygamous: one male establishes dominance over the other and monopolizes the company of the females. The *leucocephalus* mice are gregarious: the individuals live harmoniously together. The behavior of *albifrons* is intermediate. Selected color strains of *blandus* tend to pair assortatively.

In the experimental society *leucocephalus* exhibits strong sexual isolation from *blandus.* However, *albifrons* is less strongly isolated from *blandus*

than is *leucocephalus*. The F_1 hybrids between *leucocephalus* and *blandus* and between *albifrons* and *blandus* show no sexual isolation from the parent *blandus*. The hybrids are, on the other hand, sexually isolated from the parent *leucocephalus* or *albifrons*, although this isolation is less effective than that between the pure, parental stocks. It is suggested that if hybridization between these forms should ever take place in nature differential sexual isolation might operate to modify one of the forms without any reciprocal modification of the other.

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THE INHERITANCE OF EYE COLOR IN MAN—BROWN AND NONBROWN

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INTRODUCTION

THIS report is concerned with the inheritance of brown and nonbrown pigment in human eyes. No attempt is made to analyze the several eye-color patterns nor is any distinction made between the different shades of brown. The study is quantitative only insofar as quantity may be appraised by visual techniques without the aid of specialized measuring tools. The investigation was supported and the report is here published through aid from the Horace H. Rackham School of Graduate Studies, University of Michigan.

METHODS

All observations of eye color were made by myself in natural light. Although the light intensity varied considerably from day to day, the lighting generally was good. The amount of iris pigment was classified according to the following grades: 1, no brown pigment observable; 2, a trace of brown; 3, one-fourth brown; 4, one-half brown; 5, three-fourths brown; 6, a trace of nonbrown; and 7, completely brown. The analysis has given each of these grades equal rank even though this introduces a small error, since the difference (observationally) between grades 1 and 2 and between grades 6 and 7 is less than the difference between the other grades.

The data have been gathered from 107 families with a total of 212 children: (*a*) one child, forty-six families; (*b*) two children, thirty-nine families; (*c*) three children, twelve families; (*d*) four children, three families; (*e*) five children, four families; (*f*) six children, two families; and (*g*) eight children, one family. The racial ancestry of all families may be classified as North European. The family residence now is mainly in the central to east section of the Lower Peninsula of Michigan.

ANALYSIS OF DATA

The frequencies for the various eye-pigment grades in parents and in their children are given in Table I. The male children slightly outnumber the female children. Female parents are significantly darker eyed than are male parents (average grade ♀ = 3.32, σ1.84; ♂ = 2.73, σ1.67) and are somewhat more variable in eye color. Here σ refers to the standard deviation of the distribution: $\sigma^2 = 1/n\ S(x-\bar{x})^2$. Male children are darker eyed and more variable than are female children (♂ = 2.72, σ1.70; ♀ = 2.42, σ1.51), and the children are lighter eyed and less variable than the parents. These sexual differences are not consistent and probably are ascribable to the sampling process rather than to genetic peculiarities.

The mating combinations (P × P) and the grades of eye color in children (F) for each possible mating type are summarized in Table II. In terms of the mating possibilities presented by the population there is no evident homogamy in eye color. A person with a certain grade of eye color is no more likely to mate with a person having his own grade than with one having any other grade. This is shown both by the correlation coefficient ($r_{♂♀} = -.009$) and by the chi-square test ($\chi^2 = 27.82$; not significant for 36 degrees of freedom).

Among the children the statistical number of sibling pairs is 191; forty of these are female pairs, fifty-seven are male pairs, and ninety-four are male and female pairs. This statistical number is the sum of all possible sibling comparisons within a family. For example, in a family containing two male and two female children there are four male and female pairs, one male pair, and one female pair. One of the female pairs appears to be identical (Table III, Family 26). No other multiple births are recorded.

The parents range from twenty-two to fifty-four years of age and the children from three to twenty-eight years. There is no evident association between amount of brown pigment in the eye and the age of either parents ($r = .004$) or children ($r = -.074$). Further consideration of age will consequently be omitted.

The association between sex and amount of brown pigment in the iris is not significant among parents ($\chi^2 = 7.97$; P = .30) or among children ($\chi^2 = 5.40$; P = .50).

Within a family the following twelve statistical pairings may be tested. These pairings may be measured by the product-moment correlation (r), by the chi-square goodness of fit test (χ^2), by the probable significance (P), and by two additional statistics derived from the chi-square summations —mean square contingency (ϕ^2) and coefficient of mean square contingency (C).

1. ♂ parent—♀ parent. In these data there is no reported evidence of consanguinity between parents. In addition, insofar as may be determined from other measurements made on the families, such as blood

TABLE I

Distribution of Brown Series Eye Pigment in 107 Families

	Grades													
	1		2		3		4		5		6		7	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Parents														
Male	29	27.10	30	28.04	14	13.08	19	17.76	9	8.41	2	1.87	4	3.74
Female	17	15.89	34	31.78	15	14.02	14	13.08	12	11.21	5	4.67	10	9.35
Children														
Male	33	30.00	32	29.09	12	10.91	9	8.18	17	15.45	4	3.64	3	2.73
Female	33	32.35	34	33.33	14	13.73	9	8.82	6	5.88	4	3.92	2	1.96

group, secretor and taste factors, traits of the dento-facial complex, etc., there is no evidence in any instance that legal parents are not the biological parents. The parents may be assumed to be unrelated and, as pointed out above, they show no likenesses in brown pigment in the iris that are not attributed to the sampling process.

2. ♂ parent—♂ offspring. The number of pairings is 110, $r = .673$, $\chi^2 = 132.60$, $P = .01-$, $\phi^2 = 1.1839$, and $C = +.736$. Four items in the father-son pairings appear unduly to magnify the χ^2 value. If these are extracted from the manifold table, χ^2 is reduced to 78.31, C to +.648, and r to .564; P remains significant at the .01– level.
3. ♂ parent—♀ offspring. The number of pairings is 102, $r = .462$, $\chi^2 = 72.73$, $P = .01-$, $\phi^2 = .7130$, and $C = +.645$. One item is questionably erratic. Its removal reduces χ^2 to 58.00, C to +.623; r and P are not changed. The internal evidence from the correlation matrix indicates that r would be increased to about +.51 if a larger sample were used.
4. ♂ parent—offspring. There are 212 pairings, $r = .541$, $\chi^2 = 152.74$, $P = .01-$, $\phi^2 = .7205$, and $C = +.647$. Extraction of two items reduces χ^2 to 123.29 and C to .628; P remains at .01–. The value of r adequately describes the correlation matrix.

The analysis of amount of iris pigment in the male parent and in his offspring, treated both dependently and independently of sex, shows similarity which cannot be attributed to chance. Differences in the amount of similarity between father-son and between father-daughter are suggested by r, χ^2, and C. Detailed examination of the manifold tables and correlation matrices indicates that the differences present may well be attributed to minor erraticisms within the samples. A reserved conclusion, then, is that the male parent's contribution to the eye color of his offspring is probably not differentiated according to sex.

5. ♀ parent—♂ offspring. There are 110 pairs, $r = .207$, $\chi^2 = 71.71$, $P = .01-$, $\phi^2 = .6460$, and $C = +.627$. The manifold table presents no irregularities which unduly influence χ^2 and its derived statistics. The correlation value given needs no correction.
6. ♀ parent—♀ offspring. The number of pairs is 102, $r = .291$, $\chi^2 = 54.37$, $P = .02$, $\phi^2 = .3477$, and $C = +.522$. Detailed examination of the manifold tables and correlation matrices suggests no changes in the values given.
7. ♀ parent—offspring. The number of associations is 212, $r = .242$, $\chi^2 = 79.55$, $P = .01-$, $\phi^2 = .3752$, and $C = +.522$. No distributional irregularities are evident.

The P values, consistently below the .01 level, indicate the associations in eye pigment between mother-son, mother-daughter, and mother-offspring to be real and not attributal to the operation of chance factors. The difference between the mother-son (.207) and mother-daughter (.291) correlations is too small to warrant the conclusion of differential influence of sex, but is sufficiently large to suggest further consideration with more adequate data. These differences shown by the correlation are, however, contraindicated by χ^2 and C.

A comparison of the male parent-child associations with the female parent-child ones shows the former to be greatly in excess over the latter; the compared averages are: $r = .559$ and .247, $\chi^2 = 119.37$ and 68.54, and $C = .676$ and .580, respectively. Two explanations may be offered: (*a*) the results are peculiar to this sample and would not be substantiated by a more extensive body of data; or (*b*) the statistical treatment involved in setting up parent-child pairs according to the number of children in the family may magnify or minimize unduly the true differences.

The first suggestion seems most reasonable. Reference to Table II shows very clearly that the division of the total sample into its several mating combination subgroups gives a series of very small samples no one of which is sufficiently large to permit highly refined appraisal. The largest subgroup, $P_2 \times P_2$, is represented by fifteen families containing only twenty-nine children, fourteen male and fifteen female. Furthermore, twenty-one of the possible twenty-eight $P \times P$ subgroups include five or fewer families each with nine or fewer children.

The second proposition has some merit. The discussion already has shown that in a body of data of this size the method of analysis permits a very small percentage of apparently irregular items to influence strongly the magnitude of r, χ^2, and C. Aside from these difficulties there is no reason why the pairing method should, in itself, produce inaccurate results. In other words, care must be exercised when using statistics derived from small samples, regardless of the value of P.

8. Midparent—offspring. The midparent as used in this discussion is obtained by taking one-half of the sum of the grades for the male parents and the female parents. There are 212 pairs, $r = .590$, $\chi^2 = 206.75$, $P = .01-$, $\phi^2 = .9752$ and $C = +.703$. Removal of two irregular items in the manifold table reduces χ^2 to 149.53 and C to .655; P remains significant at the .01 level, and r is unaffected. There is no important difference between the midparent-male offspring and the midparent-female offspring correlations ($r = .585$ and .595 respectively).

   With the exception of the male parent-female offspring correlation, the midparent-offspring correlations are the highest of the intrafamilial pairings tested. This suggests a blending type of inheritance in the eye pigmentation.

9. ♂ sibling—♂ sibling. There are 57 pairs; $r = .490$, $\chi^2 = 67.33$, $P = .01-$, $\phi^2 = 1.1812$, and $C = +.736$. One item is irregular. Its removal reduces χ^2 to 49.27 and C to .668; P remains at less than .01, and r is unaffected.
10. ♂ sibling—♀ sibling. There are 93 pairs, and all associations except that

attributal to four erratic items is of zero order. This result is unexpected and seems to be due to the sample used. At least, the findings should be verified by additional data before attempting explanation.

11. ♀ sibling—♀ sibling. There are 40 of these associations; $r = .498$, $\chi^2 = 76.78$, $P = .01-$, $\phi^2 = 1.9195$, and $C = +.811$. Removal of one irregular item reduces χ^2 to 45.42 and C to .647; r and P are not affected.
12. Sibling—sibling. There are 191 pairs; $r = .275$, $\chi^2 = 77.33$, $P = .01-$, $\phi^2 = .4049$, and $C = +.535$. No items are strikingly erratic, and the values given reflect largely the failure of association in ♂–♀ sibling pairs.

DISCUSSION

Two statistical methods for measuring association of intrafamilial pairs have been employed; product-moment correlation and the chi-square goodness of fit. Both techniques show within-family associations that are not attributal to chance. The correlations vary between −.048 and +.673 and

TABLE II

GRADES OF BROWN AND NONBROWN EYE COLOR IN CHILDREN OF 107 FAMILIES
NUMBER OF CHILDREN OF EACH GRADE FROM PARENTS OF EACH POSSIBLE MATING TYPE

Grades of Parents	Families		Grades of Eye Color of Children							
	Number	Per Cent	1	2	3	4	5	6	7	Average
1×1	6	5.61	17	1	1	...	...	...	...	1.16
1×2	10	9.35	13	11	2	...	...	...	...	1.58
1×3	4	3.74	3	3	3	...	...	...	...	2.00
1×4	9	8.41	9	5	...	2	2	...	...	2.06
1×5	4	3.74	2	3	...	...	1	...	...	2.17
1×6	3	2.80	2	4	...	...	1	...	...	2.14
1×7	4	3.74	...	4	...	1	...	...	1	3.17
2×2	15	14.02	9	17	1	1	1	...	...	1.90
2×3	7	6.54	1	5	7	...	...	...	...	2.54
2×4	9	8.41	4	2	3	4	3	2	1	3.52
2×5	4	3.74	...	2	3	1	1	...	...	3.14
2×6	2	1.87	...	...	2	1	...	...	...	3.33
2×7	2	1.87	2	1	...	...	...	2	...	3.20
3×3	3	2.80	...	...	...	2	1	...	...	4.33
3×4	4	3.74	1	...	2	3	1	1	...	3.75
3×5	5	4.67	1	3	2	1	...	...	...	2.43
3×6	0	....	...	...	...	...	...	...	...	....
3×7	3	2.80	1	1	...	...	2	...	1	4.00
4×4	2	1.87	...	1	...	1	1	1	...	4.25
4×5	4	3.74	1	...	...	...	6	...	...	4.43
4×6	1	0.93	...	...	...	...	1	1	...	5.50
4×7	2	1.87	...	1	...	1	...	...	...	3.00
5×5	1	0.93	...	1	...	...	...	1	...	4.00
5×6	0	....	...	...	...	...	...	...	...	....
5×7	2	1.87	...	1	...	...	2	...	1	4.75
6×6	0	....	...	...	...	...	...	...	...	....
6×7	1	0.93	...	...	...	...	...	...	1	7.00
7×7	0	....	...	...	...	...	...	...	...	....
Totals	107	99.99	66	66	26	18	23	8	5	2.58

average +.491; all but one is significant at the .01 level. Considerable differences are found between the several correlations, most striking of which is the zero order correlation between male and female siblings and the marked difference between the male parent-offspring and the female parent-offspring correlations. It seems most reasonable, at the moment, to assign these differences to peculiarities of the sample rather than to set up genetic hypotheses in explanation. The midparent-offspring correlations suggest a blending type of heredity to be operative, partly at least, in the production of eye color. There is no evidence in the correlations suggestive of sex linkage, of maternal influence (contrariwise paternal influence is suggested), or of the dominance of brown over nonbrown. The analysis of association by the chi-square method substantiates the findings of correlation. Differences between χ^2 values and the derived C values are less striking than those of correlation.

The question of dominance of brown over nonbrown may be tested by examining the offspring of completely brown-eyed parents. Fourteen parents, four male and ten female, rated grade 7, were paired in the seven possible mating combinations. In the first group (PP = 1 × 7) three of the brown-eyed parents are females and one a male. All of the male offspring, three at grade 2, one at grade 4, and one at grade 7, have brown-eyed mothers; the female offspring of grade 2 has a brown-eyed father. Two families are shown with parentage 2 × 7; in one the father is graded 7 and his son 6; in the other the mother is at 7, two sons at 1, one son at 2, and one daughter at 6. Three families containing four male and one female children have parents 3 × 7. In one family father and son are 7, in another the mother is 7 and the daughter 5, and in the last the mother is 7 with three sons at grades 1, 2, and 5, respectively. In the 4 × 7 matings the female parent is at 7 in each case and one male offspring is at 2 and one female offspring at 4. There are two two-child families with parentage 5 × 7. In the first the father is brown-eyed with daughters grading 2 and 7 and in the second the mother is brown-eyed with sons at 5. Mother and son are grade 7 in the only family of parentage 6 × 7.

There can be no doubt, in view of the distribution of brown pigment among children who have one brown-eyed parent, that there is no simple dominance of brown over nonbrown in the inheritance of human eye color.

From the details cited above, and further verified by an examination of the complete data presented in Table III, it is equally clear that the postulation of sex linkage, of sex influence, or of maternal influence is not warranted.

No further analysis needs be presented to test a single gene hypothesis. Examination of Table II fails to reveal any evidence of segregation in any systematic proportion such as would be necessary to support a single gene proposition. One may postulate with assurance that the inheritance of brown versus nonbrown eye color is controlled by multiple factors and leave for further research the ascertainment of the number and mode of operation of the several genes involved.

TABLE III

Details of Eye-color Grade for Brown in Parents and Offspring Arranged by Families According to Grades of Parents

The age in years follows the eye-color grade of each child; A indicates Adult

Heredity Clinic Number	Father	Mother	Sons	Daughters
32	1	1	1(13)	
43	1	1		1(9)
......	1	1		1(11) 1(3)
1	1	1	1(19) 1(17) 1(15) 1(10)	3(21) 2(13)
72	1	1	1(11) 1(3)	1(14)
22*a*	1	1	1(A) 1(A)	1(A) 1(A) 1(A) 1(A)
40	1	2		2(13)
16*a*	2	1	1(A)	
54	2	1	2(19)	
59	1	2		1(18)
7	1	2	1(12)	1(15) 1(15)
16*b*	1	2	2(10)	2(8)
......	1	2		1(13) 2(11)
76	1	2	1(9)	2(5)
65	2	1	1(13)	2(12) 2(10) 2(8) 2(6)
68	1	2	2(18) 1(15) 2(14) 1(12) 1(9) 2(7)	3(11) 2(4)
30	3	1	3(10)	
......	1	3		1(18)
19	3	1	2(9) 1(5)	
22*b*	1	3	1(A) 3(A) 2(A) 3(A)	2(A)
39	4	1		2(12)
46	1	4	4(13)	
93	4	1		4(8)
15	1	4	2(8)	2(11)
......	4	1	2(14)	1(A)
......	1	4	1(9)	1(5)
22*c*	1	4	1(3)	1(5)
73	4	1	5(17) 5(14)	1(10) 1(4)
70	1	4	2(12)	1(9) 1(5)
83	5	1	5(12)	
327	1	5	1(9)	
111	1	5		2(9) 2(7)
78	1	5	2(13)	1(9)
3	1	6	1(10) 2(7)	5(9)
......	1	6	2(13)	1(8)
26	1	6		2(11) 2(11)
35	7	1		2(10)
37	1	7	7(13)	
107	1	7	2(7)	4(5)
......	1	7	2(11) 2(2)	
47	2	2	1(11)	
49	2	2		2(11)
51	2	2		2(11)
45	2	2		2(12)
86	2	2		2(7)
20	2	2	1(8) 2(3)	
14	2	2	1(11) 2(10)	
11	2	2	1(11)	1(13)
9	2	2	2(11)	2(4)

TABLE III—(*Cont.*)

Heredity Clinic Number	Father	Mother	Sons	Daughters
24	2	2	2(10)	5(8)
80	2	2	2(11)	1(9)
2	2	2	2(12)	2(18) 3(16) 2(14)
......	2	2	1(10) 2(5) 1(3)	
62	2	2		4(10) 2(6)
66	2	2	2(11)	1(13) 2(10)
34	3	2		3(11)
42	2	3		3(8)
91	2	3	3(12)	
99	2	3	3(9)	
60	3	2	2(10)	2(8)
23	3	2		2(9) 2(7)
63	2	3	3(4)	2(12) 3(10) 3(8) 1(6)
31	2	4	1(11)	
56	4	2	2(11)	
57	4	2	6(14)	
6	2	4	2(7)	5(10) 4(5)
12	4	2	3(9)	4(7)
8	2	4	4(14)	7(12)
......	4	2	4(8) 5(6)	1(11) 1(3)
......	4	2	3(9) 3(7) 5(6)	
75	4	2		6(18) 1(13)
29	5	2	5(14)	
33	5	2	3(10)	
......	2	5		2(13) 2(9)
79	2	5		3(13) 4(11) 3(8)
38	2	6		2(12)
21	2	6	3(13)	4(15)
28	7	2	6(9)	
67	2	7	2(8) 1(7) 1(3)	6(6)
48	3	3	5(9)	
61	3	3	4(11)	
88	3	3		4(11)
90	4	3		3(10)
27	4	3	5(14) 4(12)	1(8)
17	4	3	3(6)	6(9)
80	3	4	4(9) 4(4)	
50	3	5		1(6)
53	5	3		4(11)
55	5	3		2(13)
......	3	5		3(20)
4	3	5	2(15) 2(13)	3(11)
......	3	7		5(14)
64	7	3	7(14)	
5	3	7	1(10) 2(8) 5(6)	
13	4	4	6(12) 4(7)	
......	4	4	5(11)	2(9)
41	5	4		5(13)
92	4	5		1(9)
77	4	5	5(8) 5(5)	
71	5	4	5(14) 5(9)	5(11)

TABLE III—(*Cont.*)

DETAILS OF EYE-COLOR GRADE FOR BROWN IN PARENTS AND OFFSPRING ARRANGED BY FAMILIES ACCORDING TO GRADES OF PARENTS

Heredity Clinic Number	Father	Mother	Sons	Daughters
69	6	4	5(12) 6(10)	
44	4	7	2(13)	
85	4	7		4(10)
18	5	5	2(5)	6(9)
82	7	5		2(12) 7(8)
25	5	7	5(13) 5(10)	
36	6	7	7(13)	

SUMMARY

The inheritance of brown and nonbrown pigment has been studied quantitatively insofar as quantity may be appraised by visual techniques without the aid of specialized measuring tools.

The data were obtained from 107 families with a total of 212 children. The racial ancestry may be classified as North European.

The method of analysis was statistical, using correlation and χ^2 as the basic techniques.

The many sampling irregularities indicate the need for more extensive data.

A multiple factor type of inheritance of brown versus nonbrown eyecolor is indicated.

Dominance of brown over nonbrown pigment, sex linkage, and sex influence are contraindicated.

CONTRIBUTIONS FROM THE LABORATORY OF VERTEBRATE BIOLOGY

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VARIATION OF THE DEER-MOUSE (*PEROMYSCUS MANICULATUS*) IN PARTS OF IDAHO, MONTANA, UTAH, AND WYOMING

BY LEE R. DICE

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INTRODUCTION

THE stocks of deer-mice described in this report are the laboratory-bred descendents of animals taken at localities scattered over an area of considerable geographic extent in the northern Rocky Mountains region. The collecting stations lie in Idaho, Montana, Utah, and Wyoming. This area is highly diversified ecologically and includes such vegetational types as grassy plains, sagebrush-covered canyons, lowland conifer forest, and timberline forest. It is not surprising, therefore, that the deer-mice living in the region exhibit considerable geographic variability in their body dimensions and pelage color.

ACKNOWLEDGMENTS

The stock of deer-mice from Arco was collected by Philip M. Blossom. Olaus Murie collected the stocks from Bear Lake and Logan Canyon. The stock from Flat Creek was secured by Adolph Murie. All the other stocks were secured by me in 1933 while on an expedition sponsored jointly by the Carnegie Institution of Washington and the Museum of Zoology of the University of Michigan. The animals were reared and cared for and the study was completed in the Laboratory of Vertebrate Biology.

METHODS

All the mice herein described were born in the laboratory. After being reared under standard conditions to an approximate age of one year they were killed by ether and prepared as specimens (Dice, 1932: 5–7, 19–22). The body measurements were all made by myself. The measurements of the skull and femur and also the color readings have been made by various assistants. Donald M. Clarke carried out the statistical calculations.

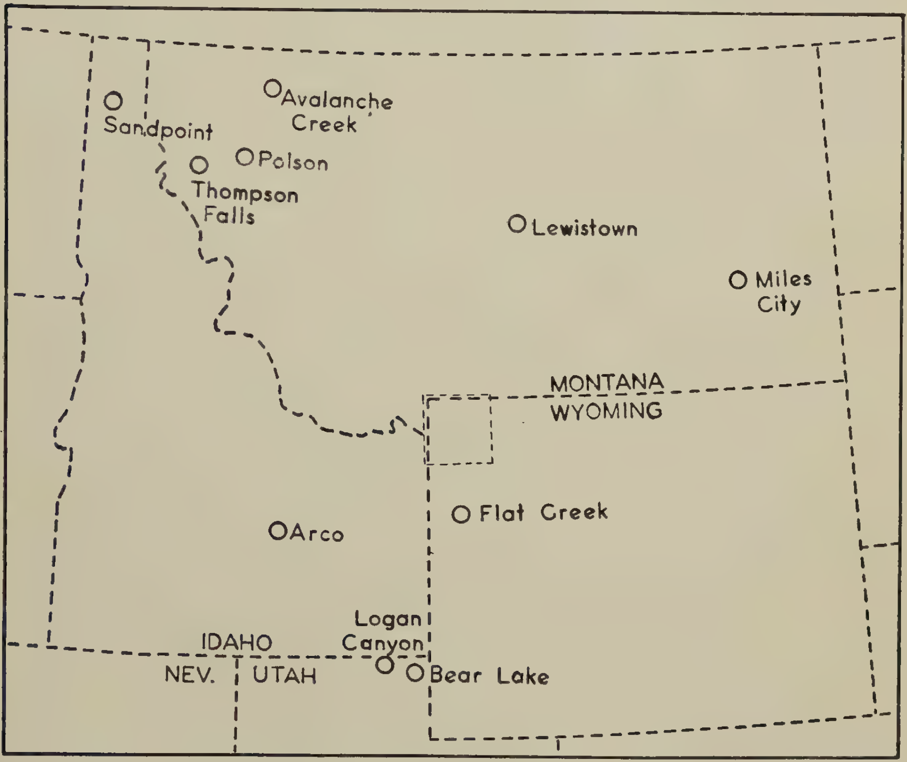
The importance of the difference between any two comparable means is usually evaluated statistically by use of the standard errors. This method, however, suffers from the difficulty that the magnitude of the standard error changes with the size of the sample. If the samples are made very large, practically every animal population can be shown to differ significantly from every other population. The difference between the means of two populations, for any particular measurement, might, therefore, be statistically significant when it is so small as to be of negligible ecological importance.

The standard error is nevertheless a useful statistic for comparing the means of the *Peromyscus* measurements. The animals included in each laboratory stock usually number between twenty-five and one hundred, and the standard errors of the several stocks, therefore, are roughly of about the same order of magnitude. In my studies of variation in *Peromyscus* I have employed a rather high standard of significance when making comparisons between means. No difference between two means that is less than 2.7 times the standard error of their difference is considered to be significant (Dice and Leraas, 1936). This procedure minimizes to some degree minor differences between the means.

Significance, used in a statistical sense, however, is only a measure of the probability that the difference between the means compared is a deviation that is not due to chance, and it is not a measure of the degree of divergence between the populations under consideration. To measure the degree of divergence between populations for any particular measured character, the standard deviation may be employed. This statistic has the advantage that it remains practically constant no matter how many individuals are included in the sample of the population from which it is derived.

In a symmetrical frequency curve 68.3 per cent of the measurements of any population should fall within one standard deviation above or below the mean. Conversely, 31.7 per cent of the measurements will fall farther from the mean than one standard deviation. Half of these measurements may be expected to be above the mean and half below it. Accordingly, slightly less than 16 per cent of the measurements will be greater than the mean plus one standard deviation and slightly less than 16 per cent will be less than the mean minus one standard deviation.

If for any given measurement the mean of one population plus once its standard deviation should be exactly equal to the mean minus once the standard deviation of another population, then only 16 per cent of each population would overlap in the resulting frequency curve. This curve would, of course, be bimodal, and 84 per cent of each of the two populations could be separated from the other on the basis of this measurement (Hubbs and Perlmutter, 1942). Should the means of the two populations compared differ by an amount greater than the sum of their respective standard deviations, the amount of overlap of the two curves will be less than 16 per cent. The standard deviation when used in this way, therefore, gives a valuable measure of the degree of divergence between populations. A precise estimate of the amount of overlap between any two populations may if desired, be calculated from the two standard deviations, but this will seldom be necessary.



MAP 1. Part of the northern Rocky Mountains region, showing collecting localities for the stocks of deer-mice.

COLLECTING STATIONS

Breeding stocks of deer-mice for this study were secured at the following localities (Map 1).

Arco. On the cinder-covered slopes of Grassy Cone, at the northwest boundary of the Craters of the Moon National Monument, twenty-six miles southwest of Arco, in Butte County, Idaho.

Avalanche Creek. In heavy cedar forest at Avalanche Creek Camp Ground, Glacier National Park, fifteen miles northeast of Belton, in Flathead County, Montana.

Bear Lake. In a short, steep canyon four miles east of the southern end of Bear Lake, in Rich County, Utah.

Flat Creek. At an elevation of about 9500 feet, near timberline, on the divide between Flat Creek and Granite Creek, nine miles east of Jackson, in Teton County, Wyoming.

Lewistown. Two and a half miles west of Lewistown, in Fergus County, Montana.

Logan Canyon. In Logan Canyon, twenty miles by road from Logan, in Cache County, Utah.

Miles City. Along Spring Creek, thirteen miles by road east of Miles City, in Custer County, Montana.

Polson. Along the shore of Flathead Lake, just west of Polson, in Lake County, Montana.

Sandpoint. Four miles south of Sandpoint, in Bonner County, Idaho.

Thompson Falls. In the valley of the Clark Fork of the Columbia four miles northwest of Thompson Falls, in Sanders County, Montana.

VARIATION IN SIZE

The greatest difference in dimensions between the ten laboratory-bred stocks of deer-mice here compared is in length of tail (Table I). The Avalanche Creek, Sand Point, and Thompson Falls stocks have relatively long tails, and the Arco, Lewistown, and Miles City stocks have short tails. The mean of this measurement for any one of the three long-tailed stocks exceeds the mean of any one of the short-tailed stocks, with one exception, by an amount greater than the sum of the standard deviations of the two stocks compared. The difference between the Thompson Falls and Arco stocks (9.73 mm.) is slightly less than the sum of their standard deviations (9.86 mm.). The differences in means between the long-tailed and short-tailed groups of deer-mice are very striking. The Avalanche Creek stock, which has the highest mean for this measurement, averages 81.10 millimeters in tail length, and the Miles City stock, which has the shortest tail, averages only 60.94 millimeters. The Miles City mice, however, have a distinctly shorter tail than the other mice of the short-tailed group, both those from Lewistown, which have a mean tail length of 64.63 millimeters, and those from Arco, with a mean of 68.51 millimeters. The remaining four stocks, those from Polson, Flat Creek, Logan Canyon, and Bear Lake, are intermediate in tail length.

The ears of these stocks of deer-mice also exhibit important differences in size. The stocks from Polson, Avalanche Creek, Thompson Falls, and Sand Point average longest in ear length, and those from Miles City and Lewistown average shortest. The differences in this dimension between the members of the large-eared and small-eared groups are greater than the sum of the respective standard deviations of any two means that may be compared. The ear length of the Polson mice, which are largest in this measure-

TABLE I

MEASUREMENTS OF *Peromyscus maniculatus* FROM THE NORTHERN ROCKY MOUNTAINS AREA
Means, standard errors, and standard deviations for 1-year age class, both sexes, in millimeters

Stock	No.	Mean Age in Weeks	No. of Parents	Body Length	Tail Length	Hind-foot Length	Ear Length
artemisiae							
Avalanche Creek	42	54	5	97.83 ± .56 (3.66)	81.10 ± .64 (4.13)	20.800 ± .080 (.518)	18.759 ± .112 (.697)
Flat Creek	80	45	16	95.94 ± .48 (4.32)	71.38 ± .68 (6.09)	20.441 ± .057 (.514)	17.994 ± .080 (.718)
Polson	79	55	5	95.13 ± .47 (4.12)	72.67 ± .54 (4.54)	20.173 ± .075 (.660)	18.812 ± .077 (.673)
Sand Point	32	55	4	96.84 ± .69 (3.87)	80.29 ± .74 (4.12)	20.278 ± .109 (.615)	18.494 ± .094 (.532)
Thompson Falls	97	54	10	97.97 ± .41 (3.99)	78.24 ± .53 (5.06)	20.670 ± .058 (.567)	18.716 ± .024 (.234)
osgoodi							
Lewistown	61	53	5	100.08 ± .53 (4.12)	64.63 ± .67 (5.21)	19.998 ± .086 (.667)	17.063 ± .058 (.447)
Miles City	69	57	6	95.10 ± .46 (3.83)	60.94 ± .41 (3.40)	20.137 ± .063 (.524)	17.326 ± .077 (.637)
sonoriensis							
Arco	115	56	13	96.08 ± .35 (3.76)	68.51 ± .45 (4.80)	20.346 ± .063 (.680)	18.361 ± .068 (.729)
Bear Lake	26	52	5	94.00 ± .79 (4.02)	69.38 ± .84 (4.11)	19.762 ± .131 (.666)	18.138 ± .141 (.717)
Logan Canyon	63	46	8	93.78 ± .47 (3.74)	71.10 ± .60 (4.75)	20.436 ± .075 (.592)	17.769 ± .078 (.616)

	Femur	Mandible	Condyle-premaxilla	Condyle-zygoma	Bullar Width
artemisiae					
Avalanche Creek	15.993 ± .090 (.576)	16.602 ± .055 (.358)	24.939 ± .075 (.482)	17.940 ± .054 (.340)	11.110 ± .031 (.196)
Flat Creek	16.254 ± .070 (.614)	16.207 ± .047 (.410)	24.661 ± .059 (.519)	17.505 ± .048 (.417)	11.012 ± .027 (.241)
Polson	15.769 ± .092 (.716)	16.192 ± .061 (.474)	24.588 ± .088 (.678)	17.519 ± .067 (.513)	11.115 ± .035 (.276)
Sand Point	15.913 ± .092 (.516)	16.069 ± .069 (.390)	24.890 ± .092 (.514)	17.716 ± .059 (.331)	10.819 ± .038 (.217)
Thompson Falls	16.247 ± .071 (.689)	16.549 ± .048 (.467)	24.946 ± .069 (.680)	17.711 ± .047 (.456)	11.028 ± .024 (.233)
osgoodi					
Lewistown	16.503 ± .085 (.653)	16.163 ± .053 (.405)	24.561 ± .094 (.725)	17.703 ± .068 (.520)	10.983 ± .035 (.270)
Miles City	15.978 ± .082 (.681)	16.370 ± .046 (.388)	24.352 ± .064 (.529)	17.726 ± .045 (.368)	11.036 ± .029 (.240)
sonoriensis					
Arco	16.061 ± .060 (.643)	15.938 ± .039 (.420)	24.188 ± .060 (.641)	17.177 ± .042 (.451)	10.859 ± .024 (.254)
Bear Lake	16.196 ± .120 (.598)	16.084 ± .083 (.413)	24.452 ± .107 (.535)	17.472 ± .071 (.353)	10.948 ± .040 (.202)
Logan Canyon	16.152 ± .075 (.589)	15.858 ± .047 (.368)	24.171 ± .068 (.539)	17.059 ± .041 (.329)	10.751 ± .029 (.232)

ment, averages 18.812 millimeters, and that of the Lewistown mice, which are smallest, averages 17.063 millimeters.

None of the other body measurements and none of the measurements of the skull or femur differ between the several stocks in any consistent and important way. Only in length of mandible and in the condylo-zygomatic measurement of the skull are the means of the Avalanche Creek mice greater than those of the Logan Canyon mice by amounts that are greater than the sums of the two respective standard deviations.

TABLE II

PELAGE COLOR OF *Peromyscus maniculatus* FROM THE NORTHERN ROCKY MOUNTAINS AREA

Mean tint-photometer readings, standard errors, and standard deviations for 1-year age class, both sexes, in per cent

Stock	Dorsal Stripe		
	Red	Green	Blue-violet
artemisiae			
Avalanche Creek	8.21 ± .20 (1.26)	6.12 ± .15 (.98)	4.76 ± .13 (.84)
Flat Creek	9.60 ± .23 (2.06)	6.94 ± .18 (1.81)	5.19 ± .14 (1.57)
Polson	9.56 ± .12 (1.08)	7.58 ± .11 (.98)	5.84 ± .10 (.90)
Sand Point	7.64 ± .28 (1.56)	6.00 ± .22 (1.24)	4.68 ± .17 (.96)
Thompson Falls	7.65 ± .12 (1.21)	5.88 ± .10 (1.02)	4.47 ± .09 (.84)
osgoodi			
Lewistown	10.27 ± .17 (1.34)	7.85 ± .16 (1.28)	5.67 ± .13 (1.04)
Miles City	13.54 ± .27 (2.23)	10.37 ± .21 (1.77)	7.89 ± .17 (1.41)
sonoriensis			
Arco	9.51 ± .17 (1.83)	7.34 ± .14 (1.46)	5.51 ± .11 (1.11)
Bear Lake	12.27 ± .27 (1.52)	9.33 ± .16 (.91)	7.09 ± .17 (1.00)
Logan Canyon	11.68 ± .20 (1.54)	8.97 ± .17 (1.30)	7.08 ± .17 (1.28)
	Side Stripe		
artemisiae			
Avalanche Creek	16.07 ± .31 (2.01)	11.93 ± .26 (1.67)	9.14 ± .23 (1.51)
Flat Creek	18.95 ± .21 (1.86)	13.68 ± .17 (1.89)	9.84 ± .15 (1.47)
Polson	17.43 ± .24 (2.26)	13.86 ± .21 (1.93)	11.23 ± .19 (1.75)
Sand Point	15.65 ± .32 (1.79)	11.58 ± .22 (1.24)	9.26 ± .20 (1.11)
Thompson Falls	14.96 ± .19 (1.91)	11.37 ± .15 (1.46)	8.62 ± .12 (1.18)
osgoodi			
Lewistown	19.72 ± .34 (2.66)	14.55 ± .28 (2.20)	11.10 ± .24 (1.85)
Miles City	24.83 ± .39 (3.25)	18.81 ± .27 (2.27)	14.26 ± .22 (1.83)
sonoriensis			
Arco	18.61 ± .24 (2.58)	14.22 ± .19 (1.97)	10.98 ± .15 (1.60)
Bear Lake	22.94 ± .40 (2.32)	18.00 ± .33 (1.92)	13.55 ± .32 (1.83)
Logan Canyon	22.43 ± .24 (1.84)	16.62 ± .22 (1.68)	12.68 ± .19 (1.50)

It will be noted that the Avalanche Creek, Sand Point, and Thompson Falls stocks are notable for both long tails and large ears, when compared to the other stocks here described. Likewise, the Miles City and Lewistown mice have both short tails and small ears. On the other hand, the Arco mice, which have fairly short tails, rank only intermediate among the other stocks in ear size. Furthermore, the Polson mice, which average large in ear size, rank only intermediate among the stocks in length of tail.

VARIATION IN PELAGE COLOR

The pelage of the Miles City, Bear Lake, and Logan Canyon mice is relatively pale compared to that of the other seven stocks. This is shown by their high average tint photometer readings. On the other hand, the Thompson Falls, Sand Point, and Avalanche Creek mice are relatively dark, as shown by low readings. The difference between any given mean color reading for any one of the pale stocks listed and the comparable mean of any one of the dark stocks is greater than the sums of the two respective standard deviations. This is true for each color screen, red, green, and blue-violet, and for both dorsal and side stripes. The other four stocks, those from Lewistown, Flat Creek, Arco, and Polson, are intermediate in shade of pelage (Table II).

TAXONOMIC RELATIONS OF THE STOCKS

The stocks of deer-mice from Sand Point, Thompson Falls, and Avalanche Creek agree in being dark in pelage and in having long tails and large ears, when compared with the other seven stocks here described. The three collecting stations named lie within the geographical range assigned to the subspecies *Peromyscus maniculatus artemisiae* (Osgood, 1909 : 58–60; Davis, 1939, Fig. 23), and their characters agree with the diagnosis of that form. Measurements of another stock of *artemisiae* from St. Mary, Glacier Park, Montana, have previously been given by Murie (1933). Murie's animals, however, were not bred in the laboratory, and his measurements, therefore, are not exactly comparable to those of the stocks here described. The St. Mary mice, nevertheless, agree with the mice here assigned to *artemisiae* in having relatively long tails, large ears, and dark pelage color.

The subspecies *artemisiae* is an inhabitant of the conifer forests of the northern Rocky Mountains in western Montana, northern Idaho, eastern Washington, and the adjacent parts of British Columbia. The type locality is Ashcroft, British Columbia. The subspecies has been identified as far south as the Blue Mountains of southeastern Washington. Measurements have previously been given of laboratory-bred stocks from Pullman, Lyons Ferry (north side of Snake River), Palouse Falls, Dayton, Hompeg Falls, and Godman Springs, in Washington, and from Troy in Idaho (Dice, 1939: 21). There are, however, considerable differences between these several stocks, and not all of them combine the characters of long tail, large ear, and dark pelage color.

The mice from Miles City differ from *artemisiae* by having relatively short tails, small ears, and pale pelage. They may be assumed to represent the subspecies *osgoodi* (Mearns, 1911), whose type locality of Calf Creek, Custer County, Montana, is in the same county as the Miles City collecting station. The subspecies *osgoodi,* which was discussed by Osgood (1909 : 75) under the name *nebrascensis,* occupies a considerable range in eastern Montana and in the neighboring states and provinces. It is in general an inhabitant of semiarid grass or sagebrush-covered plains and foothills.

Laboratory-bred stocks of deer-mice assigned to the subspecies *osgoodi* have previously been described from three localities in northeastern Utah (Leraas, 1938), from several localities in western North Dakota (Dice, 1940), from one locality in eastern Wyoming and one in western Nebraska (Dice, 1941), and from several localities in the Black Hills region of South Dakota and adjacent Wyoming (Dice, 1942). In addition, Murie (1933) has given the measurements of a stock captured alive at St. Mary, on the eastern side of Glacier Park, Montana, and reared to maturity in the laboratory. These several stocks of *osgoodi* differ considerably among themselves in dimensions and in pelage color. They, however, in general agree in having relatively short tails and pale color when compared to *artemisiae.*

The Polson locality lies within the range of *artemisiae,* though it is in a wide valley that is mostly covered by grass rather than by conifer forest. The mice average significantly smaller than the Avalanche Creek, Sand Point, and Thompson Falls stocks in tail length, but exceed those stocks slightly, though not significantly, in ear length. For neither measurement are the differences between the means as great as two standard deviations. The Polson mice on the other hand exceed the Miles City mice significantly in both tail length and in ear length, and the differences are greater than the sums of the respective standard deviations. In tint photometer readings of pelage color the Polson mice average more or less intermediate between *artemisiae* and *osgoodi.* On the basis of their dimensions and geographical origin they may be assigned to *artemisiae.* The paler color of their pelage compared to that of the mice of the other *artemisiae* stocks is perhaps correlated with the scantiness of forests in the vicinity of their collecting station.

The Lewistown locality lies on the eastern margin of the range of *artemisiae.* The situation is partly surrounded by mountains. Conifer forest here alternates extensively with grass-covered hills. The collecting station itself was in open fields. The mice of this stock average small both in tail length and ear length, and their means do not differ significantly from those of the Miles City mice. In pelage color they are somewhat intermediate between *artemisiae* and the Miles City *osgoodi,* but their tint photometer readings are closest to those of the Miles City mice. The Lewistown stock may accordingly be assigned to *osgoodi,* though it is not fully typical in color.

The Flat Creek locality is in northwestern Wyoming, south of Yellowstone Park, and considerably southeast of the main range of *artemisiae.* Osgood (1909: 61), however, assigns specimens from this region to that subspecies. The Flat Creek mice significantly exceed both the Lewistown and Miles City mice in tail length and ear length. The difference in their tail length from that of the Miles City mice exceeds the sum of the two respective standard deviations, but their difference from the Lewistown mice is not so great. The difference in ear length between the Flat Creek and the Miles City and Lewistown mice is less than two standard deviations. The Flat

Creek stock is significantly exceeded in both tail length and ear length by the four stocks of this group already assigned to *artemisiae,* though the differences between the means are not as great as the sums of the respective standard deviations. In tint photometer readings of pelage color the Flat Creek mice are intermediate between the stocks of *artemisiae* and the Miles City *osgoodi.* On the basis of their rather long tails the Flat Creek mice may be assigned to *artemisiae,* but they are not typical of that subspecies either in measurements or pelage color.

The Arco locality falls within the range of the subspecies *sonoriensis* (Davis, 1939, Fig. 23). This subspecies is said by Osgood (1909: 75, 89–94) not to differ greatly in color from *osgoodi* (there called *nebrascensis*), but to be somewhat larger in size and to have a longer tail.

The differences in dimensions between the mice of the subspecies *osgoodi* and *sonoriensis,* however, are not very conspicuous nor always consistent. Compared to a stock of undoubted *sonoriensis* from Miller Canyon, Arizona (Dice, 1938*a*), from only a short distance northeast of the type locality of that subspecies, the Miles City *osgoodi* do not differ significantly in the means of either body length or of tail length. The Miles City mice have the larger ears, the difference in means being 0.390 ± .110 millimeters, which is statistically significant. The differences between the two stocks in pelage color readings are not consistent, the Miles City mice averaging darker than the Miller Canyon mice on the dorsal stripe but paler on the side of the body. There is, therefore, no important difference either in body dimensions or in pelage color between these two laboratory-bred stocks representing, respectively, *osgoodi* and *sonoriensis.*

The Arco mice are appreciably darker than *osgoodi* from Miles City, as shown by their significantly lower tint photometer readings (Table II). The Arco locality, however, is in an area where there is a considerable amount of dark lava, and it is likely, therefore, that a dark local race of these mice has developed in that vicinity. There is no significant difference in body length between the Miles City and Arco stocks. The Arco stock significantly exceeds the Miles City stock in both tail length and ear length, though for neither measurement is the difference between the two respective means as great as the sum of their standard deviations.

Compard to *sonoriensis* from Miller Canyon, the Arco mice do not differ significantly in body length, but are significantly the larger in tail length and are considerably the darker in pelage, both on the dorsal stripe and on the side of the body. A stock of *sonoriensis* from Victorville, California (Dice, 1938*b*, Tables III and IV), significantly exceeds the Arco stock in tail length, ear length, and in readings of pelage color. This Victorville stock, however, was considerably inbred in the laboratory, and it may not well represent the wild population in that part of the Mohave Desert.

The Arco stock is therefore assigned to the subspecies *sonoriensis* because of the geographical position of the locality, rather than because of any important demonstrated difference in characters from the mice of the subspecies *osgoodi.* The Arco stock, furthermore, may be assumed to represent a dark but probably weakly differentiated local race.

The Bear Lake and Logan Canyon localities are both in the canyons of northeastern Utah, where grass and sagebrush alternate with shrubby thickets and with stands of juniper and other trees. The mice of both these stocks average more or less intermediate in tail length and ear length between *artemisiae* and the Miles City *osgoodi.* In pelage color they also are intermediate, but closest to the Miles City stock. The differences in tint photometer readings between them and the Miles City mice are statistically significant for most color comparisons when measured by the standard errors, but are always less than the sum of the two respective standard deviations. On the contrary, the differences in mean tint photometer readings between the two Utah stocks and those of the subspecies *artemisiae* from Sand Point, Avalanche Creek, and Thompson Falls are always greater than the sum of the two respective standard deviations.

Compared to a stock of laboratory-bred mice from Little Spring, Arizona (Dice, 1938*a,* Tables III and IV), which may be assumed to be the type locality of *rufinus,* the two Utah stocks average considerably longer in tail length. In ear length the Little Springs stock averages the longer, but the differences from the Utah stocks are not very great. In pelage color the Utah stocks are considerably paler than the Little Springs stock, and the differences in tint photometer readings are of high statistical significance. On the basis of pelage color, therefore, these two Utah stocks cannot be referred either to *artemisiae* or *rufinus.*

Compared to *sonoriensis* from Miller Canyon, Arizona (Dice, 1938*a,* Tables III and IV), the two Utah stocks average significantly longer in tail length and ear length. In tint photometer readings for dorsal stripe red the Utah stocks average significantly lower than the Miller Canyon stock, but there is very little difference between them in the color of the side of the body.

The Logan Canyon and Bear Lake mice are rather close to the Arco mice in their characters. They do not differ in any important way in ear length nor tail length. The Utah stocks are slightly paler than the Arco stock in pelage. The differences in tint photometer readings are statistically significant when measured by the standard errors, but are not so great as the sums of the respective standard deviations.

The Bear Lake and Logan Canyon stocks also closely resemble some of the stocks from the Uinta Mountains region, northeastern Utah, assigned by Leraas (1938) to *osgoodi.* There is, however, so much variability among the deer-mice living in this ecologically diversified region, that any precise delimitation here between the ranges of *sonoriensis* and *osgoodi* is impossible with the information at hand. Because of their relatively long tails as compared to the Miles City mice and their similarity to the Arco mice, the Logan Canyon and Bear Lake stocks are here tentatively assigned to *sonoriensis.*

DISCUSSION

In the geographical area covered by the collecting stations whence the breeding stocks for this study were secured the deer-mice are of two strikingly different types. The geographic race or subspecies called *artemisiae* is a dark, long-tailed, and large-eared form, which is mostly restricted to the conifer forests of the mountains. On the grass-covered or sage-brush-covered plains and hills most of the deer-mice are pale in pelage color and have relatively short tails and small ears. They are assigned to the two subspecies *osgoodi* and *sonoriensis.* The tail of *sonoriensis* is said to average slightly longer in length than the tail of *osgoodi,* but the differences in the characters of these two forms are not well marked.

The several laboratory-bred stocks assigned to the same subspecies often differ considerably from one another in their average dimensions and pelage color. Considerable variations from place to place in the characters of the field populations of these deer-mice are thereby indicated, even within the range of a single subspecies. A few stocks are more or less intermediate between two adjacent subspecies, and their exact subspecific assignment has had to be made more or less arbitrarily.

Where the ranges of *artemisiae* and *osgoodi* meet at the western border of the mountains at Glacier Park, Montana, these two forms do not interbreed, and they occupy different types of habitats (Murie, 1933). It is, however, not certain that the two forms are everywhere so sharply separated. It is possible that there is interbreeding between them at some places where the two forms meet north or south of Glacier Park.

The long tail of the subspecies *artemisiae* is probably an adaptation for semiarboreal life in a forest habitat. The shorter tails of the subspecies *osgoodi* and *sonoriensis* may likewise be an adaptation for cursorial life in open fields. There is no evidence at hand, however, to prove that length of tail actually is an important factor in the survival of either of these forms. Neither is there any evidence that the differences in the size of the ear that distinguish these forms are of adaptive significance.

The prevailing dark pelage of the subspecies *artemisiae* is undoubtedly correlated in general with life in heavy conifer forest. Likewise, the paler pelages of *osgoodi* and *sonoriensis* are probably correlated with life in more sparsely vegetated habitats.

The climate of the montane-forest belt that is the home of *artemisiae* undoubtedly differs in important respects from the climate of the lower belt that is the habitat of *osgoodi* and *sonoriensis.* The climate of each belt undoubtedly determines the type of vegetation that grows there. On the contrary, there is no evidence that the climate in any way directly affects the characters of the deer-mice.

The amount of humus in the soil at any given locality depends to a considerable degree upon the heaviness of the vegetative cover. It would be expected, therefore, that the surface soil would be darker in the habitat of *artemisiae* than in that of *osgoodi* and *sonoriensis.* Soil samples, unfortunately, are available from only a few of the collecting stations where the stocks of mice here described were secured. The readings of the Miles City soil sample average 21.5 per cent for reflected red, and the Thompson Falls soil averages only 10.8 per cent. The Lewistown soil, however, also reads 10.8 per cent for red, in spite of the fact that the mice from this station, though somewhat intermediate in pelage color, are assigned to *osgoodi.* The soil at the Polson station is still paler, with readings for red of 16.6 per cent, though the mice here are assigned to *artemisiae.* The soil at the Arco station averages 7.8 per cent of reflected red, this low reading being undoubtedly due to the presence of volcanic cinders. The mice of the Arco stock are somewhat darker than would be expected of *sonoriensis.* A dark-colored local race has presumably been evolved in that area, in correlation with the dark color there of the surface soil. This evidence, while not fully consistent, agrees in general with the thesis that the pelage color of the upper parts of the mice tends to vary in accordance with the color of the surface soil of their habitat (Dice and Blossom, 1937: 108).

It may be concluded that the differences in characters between the deer-mice of the subspecies *artemisiae* and those of the *osgoodi-sonoriensis* complex are correlated with differences in the climate, vegetation, and soil color of their respective habitats. We have very little precise information, however, about the manner in which any particular character of the animals is influenced by the environment, nor do we know much about how these correlations are brought about or maintained.

SUMMARY

Measurements are given of body dimensions and pelage color for laboratory-bred stocks of deer-mice from ten localities in the northern Rocky Mountains region.

The long-tailed, large-eared, and dark deer-mice of the geographic race *Peromyscus maniculatus artemisiae* are, in general, inhabitants of the montane conifer forests. The shorter-tailed, smaller-eared, and paler mice of the races *osgoodi* and *sonoriensis* live mostly on the grass-covered and sagebrush-covered plains and lower hills. The differences in the characters of these two types of deer-mice are correlated with differences in the climate, vegetation, and soil color of their respective habitats. The precise factors of environment and of organic response involved in producing these correlations are, however, largely unknown.

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CONTRIBUTIONS FROM THE LABORATORY OF VERTEBRATE BIOLOGY

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SEROLOGICAL DIFFERENCES IN THE GENUS *PEROMYSCUS* DEMONSTRABLE WITH NORMAL HUMAN SERA

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INTRODUCTION

In number of species and subspecies the rodent genus *Peromyscus* outranks all other mammalian genera of North America. Most of its species are easily reared in the laboratory, and some are known to produce fertile species hybrids under laboratory conditions (Dice, 1940). Such qualities make this group of exceptional value for the study of mammalian variation at the species and subspecies levels. A search for serological differences between various *Peromyscus* species has been initiated by Moody, using anti-*Peromyscus* immune sera of rabbits and various rodents. Differences in the serum proteins, such as were readily demonstrated between various rodent families and genera (Levine and Moody, 1939), could not be detected within the genus *Peromyscus* by means of ordinary precipitin tests. By means of the Schultz-Dale reaction, however, Moody (1940) was able to differentiate the serum of *P. maniculatus gracilis* from that of *P. gossypinus palmarius*. The same author has also reported (1941) the separation of *P. leucopus* and *P. maniculatus* by means of agglutination tests with absorbed sera of rabbits immunized against *Peromyscus* cells.

The present report deals with antigenic differences in *Peromyscus* erythrocytes which are demonstrable with normal human agglutinins. Although such preformed antibodies would not be expected to differentiate *Peromyscus* antigens as readily as would the immune antibodies employed by Moody, the results of the preliminary tests described herein suggest that considerable differentiation may actually be secured. The ready availability of human sera constitutes a decided practical advantage. Moreover, the antigens demonstrated by such sera are of added interest owing to the fact that some of them can be identified as related to the human isoagglutinogen B. Since the work of von Dungern and Hirschfeld (1911), it has been known that the B factor of human erythrocytes is represented by serologically similar substances in the red blood cells of many other mammals. Like the human group substance A, the B agglutinogen is therefore a heterophile antigen of widespread occurrence. In man, the alcohol-soluble B factor of the red blood cells is determined by a gene, which is an allele of those for the A and O substances, and a second pair of genes (the ''secretor'' and ''nonsecretor'' factors) determines its concomitant presence or absence in water-soluble form. A further study of the B-like antigens of *Peromyscus* should prove of interest in connection with the genetics and evolution of these substances.

Other human agglutinins which may serve for the separation of certain *Peromyscus* species are apparently present in most or all normal human sera, irrespective of blood group. These are therefore inactive against human red blood cells and are designated as heteroagglutinins.

The mice used in the investigation were mostly taken from stocks available in the Laboratory of Vertebrate Biology. I am especially indebted to Elizabeth Barto for collecting the blood samples and to Dr. Alexander S. Wiener, who provided the author with several testing sera and offered valuable suggestions. Paul A. Moody kindly donated several specimens of *P. gossypinus*. The study was supported in part by the Horace H. Rackham School of Graduate Studies.

MATERIALS AND METHODS

Eight species of *Peromyscus* are represented in the various tests to be described. Since F_1 hybrids of *maniculatus* and *polionotus* were available for testing, these two species were sampled somewhat more adequately than the others. The term cenospecies (Blair, 1943), used in the following list of species, corresponds essentially to the species group of previous authors.

Peromyscus

Subgenus *Haplomylomys*

eremicus cenospecies
- *P. eremicus eremicus,* from Carrizozo, New Mexico

crinitus cenospecies
- *P. crinitus crinitus,* from St. George, Utah

Subgenus *Peromyscus*

maniculatus cenospecies
- *P. maniculatus exiguus,* from San Martin Island, Baja California
- *P. maniculatus blandus,* from Alamagordo, New Mexico
- *P. maniculatus* (various subspecies, including complex hybrids of *artemisiae, bairdii, gambeli, nebrascensis, rubidus,* and *sonoriensis*)

P. polionotus leucocephalus, from Santa Rosa Island, Florida
P. polionotus (various subspecies, including complex hybrids of *albifrons, leucocephalus, polionotus,* and *rhoadsi*)
F_1 hybrids of *P. maniculatus* and *P. polionotus*
leucopus cenospecies
P. leucopus noveboracensis, from Ann Arbor, Michigan
P. gossypinus palmarius, from Seebring, Florida
truei cenospecies
P. nasutus nasutus, from Lincoln, New Mexico
P. truei truei, from Capitan Gap, New Mexico

In most cases, the samples of blood were obtained from animals which had been set aside for sacrifice. Under etherization the jugular veins were severed, and the blood was collected into small test tubes. After clotting, the cells were resuspended in saline and washed several times. In some tests, where small quantities of cell suspension were sufficient, one or two drops of blood were obtained from the tail.

Approximately 2 per cent suspensions of cells were used in the agglutination tests. One or two drops of cell suspensions were mixed with like volumes of testing fluid in 10-mm. test tubes, and the mixtures were allowed to stand at room temperature with occasional shaking. Readings were made at one hour. The agglutination reactions are recorded as –, ±, +, ++, +++, ++++, the last grade denoting a completely agglutinated cell mass that did not break up on moderate shaking. For the absorption experiments, small quantities of blood cells were measured with hemocytometer pipettes and diluted with saline as required. All absorptions were carried out for a period of one hour at room temperature. Human blood suspensions of groups O, A_1, A_2, and B, and of types M, MN, and N were employed as controls in all series of tests.

ABSORPTION EXPERIMENTS WITH GROUP-A SERA

Each sample of *Peromyscus* blood was routinely tested with a number of reagents ordinarily employed for the serological typing of human blood. These included human group-B (anti-A) sera, human group-A (anti-B) sera, and absorbed rabbit antisera specific for the human agglutinogens A, B, M, and N. The results of such simple typing tests are summarized in Table I. The extent of agglutination recorded in this table represents the average reaction obtained with the several blood specimens of each species. Some quantitative variation within species was noted, but in no case did two individuals of the same species give completely different reactions, that is, one positive and the other negative.

The blood cells of all *Peromyscus* specimens were agglutinated by all human sera with the exception of two group-B sera (B–41 and B–518), which gave negative reactions with all *Peromyscus* bloods tested (Table I). A single serum of group O and one of group AB were also used in a part of the tests (not indicated in Table I) and were likewise found to possess agglutinins against all *Peromyscus* species. The two exceptional sera, B–41 and B–518, were of unusual origin and their significance will be discussed later. These being disregarded, it may best be considered that any human serum, regardless of blood group, may possess anti-*Peromyscus* agglutinins. This being the case, it is evident that the presence of A-like or B-like antigens cannot be established by the use of untreated human sera. It is shown in the following section, however, that a B-like substance is definitely present in species *maniculatus, polionotus, leucopus,* and *gossypinus.* It is therefore of interest that the bloods of these species are not agglutinated by anti-human-B sera produced by rabbits. This result is identical with the findings of Landsteiner and Miller (1925) on the bloods of monkeys and other mammals possessing B-like factors. These authors showed that anti-human-B agglutinins of immune rabbit sera were reactive with the cells of certain (group-B) apes and men, but not with monkeys and other mammals possessing a B-like antigen. The B factor of the higher primates is thus distinguishable from that of other mammals. According to Friedenreich and With's (1933) theory on the multiplicity of B components, these facts are explained by assuming that the human B agglutinogen contains a component shared by certain apes but not by *Peromyscus* and other mammals.

TABLE I

Agglutination Reactions of the Cells of Several *Peromyscus* Species

Species	No. of Bloods Tested	Normal Human Sera			Immune Rabbit Sera			
		Group B (most)	B–41 and B–518	Group A	Anti-A	Anti-B	Anti-M	Anti-N
leucopus	3	++++	–	++++	–	–	–	–
gossypinus	2	++++	–	++++	–	–	–	–
maniculatus	17	++++	–	++++	–	–	–	–
F$_1$ *maniculatus* × *polionotus* hybrids	7	++++	–	++++	–	–	–	–
polionotus	12	++++	–	++++	–	–	–	–
nasutus	6	±	–	±	–	–	–	–
truei	3	±	–	±	–	–	–	–
eremicus	9	++	–	++	–	–	–	–
crinitus	3	+	–	+	–	–	–	–

The finding that *Peromyscus* cells are not reactive with anti-M and anti-N rabbit agglutinins also agrees with the observations of Landsteiner and Miller, whose studies indicate that these properties are possibly restricted to the blood of man, apes, and certain monkeys.

Cells of the *Peromyscus* species *maniculatus, polionotus, leucopus,* and *gossypinus* were much more strongly agglutinated by untreated human sera than were those of species *nasutus, truei, eremicus,* and *crinitus* (Table I). These results recommended the trial of absorption methods for the purpose of securing reagents which might effect a qualitative separation of certain species. One such experiment, involving the use of five human group-B and thirty-two *Peromyscus* bloods, is summarized in Table II. For this experiment, a human group-A serum of high isoagglutinin titer (1024) was selected. Parts of this serum, diluted 1:16, were absorbed with one-tenth volumes of washed, packed cells of the thirty-seven bloods. Each of these preparations was then tested against cells of the same series of bloods. The average degree of agglutination within sets of readings involving test cells and absorbing cells of the same species is shown in Table II. Variation within species was again small as compared with the differences between species. Reactions recorded in Table II as ++++ were almost all complete agglutinations; reactions recorded as – were mostly negative with an occasional trace of agglutination (±); and reactions recorded as + or ++ were somewhat more variable.

Table II confirms Table I in showing a marked contrast between the cells of *maniculatus, polionotus,* F_1 *maniculatus* × *polionotus* hybrids, and *leucopus* and those of the species *nasutus, truei, eremicus,* and *crinitus.* Group-A serum exhausted of its agglutinins against any one of the species *nasutus, truei, eremicus,* or *crinitus* will readily distinguish cells of such species from those of *maniculatus, polionotus,* or *leucopus.* Furthermore, continued treatment of group-A sera with excesses of *nasutus, truei, eremicus,* or *crinitus* cells was found to be ineffective in removing the agglutinins against the remaining species. The results also suggested that further discrimination within the group *maniculatus, polionotus,* and *leucopus* should be possible with the aid of appropriately absorbed and diluted A sera. This possibility was tested by attempting to classify a series of fourteen bloods which were known only to comprise a collection of the three species, *maniculatus, polionotus,* and *eremicus.* By using *eremicus*-absorbed and *polionotus*-absorbed A serum, thirteen of these were correctly identified, the only error consisting in classifying a *maniculatus* blood as belonging to *polionotus.*

The experiment described in Table II involved a constant ratio between the volume of serum and the volume of absorbing cells. A number of tests were next performed using varying quantities for the absorptions. This was done with the view of determining the optimum proportion of absorbing cells for the preparation of a *polionotus*-absorbed serum with which to differentiate the species *maniculatus* and *polionotus* and also to determine whether larger quantities of the cells of these mice would exhaust the serum of its anti-human-B agglutinin. Figure 1 depicts the results of one such experiment comparing the absorptive capacities of *maniculatus, polionotus,* and human group-B cells. Parts of serum A–121, diluted 1:4, were absorbed with varying volumes of cells of these three species and then titrated against cells of each of the same three bloods. The titration curves against human group-B cells show that cells of the *maniculatus* mouse removed the β agglutinin about as rapidly as did human group-B cells. Furthermore, *polionotus* blood also removed the β agglutinin, although less effectively than *manicu-*

TABLE II

Agglutination of Five Human Group-B and Thirty-two *Peromyscus* Bloods with a Human Group-A Serum, before and after Absorption with Cells of the Same Individuals

Cells	No. of Bloods Tested	Serum A–121 1: 16 Unabsorbed	Serum A–121, 1: 16, Absorbed with 1/10 Vol. Cells of:								
			B	*l*	*m*	*mp*	*p*	*e*	*c*	*n*	*t*
Human group (B)	5	++++	+	++	+	±	++++	++++	++++	++++	++++
leucopus (*l*)	2	++++	++++	–	–	±	±	++++	++++	++++	++++
maniculatus (*m*)	6	++++	++++	–	–	–	++	++++	++++	++++	++++
F_1 *maniculatus* × *polionotus* hybrids (*mp*)	4	++++	++++	–	–	–	++	++++	++++	++++	++++
polionotus (*p*)	6	++++	++++	–	–	–	–	++++	++++	++++	++++
eremicus (*e*)	6	++	++	–	–	–	–	–	–	–	–
crinitus (*c*)	5	+	+	–	–	–	–	–	–	–	–
nasutus (*n*)	2	+	+	–	–	–	–	–	–	–	–
truei (*t*)	1	±	±	–	–	–	–	–	–	–	–

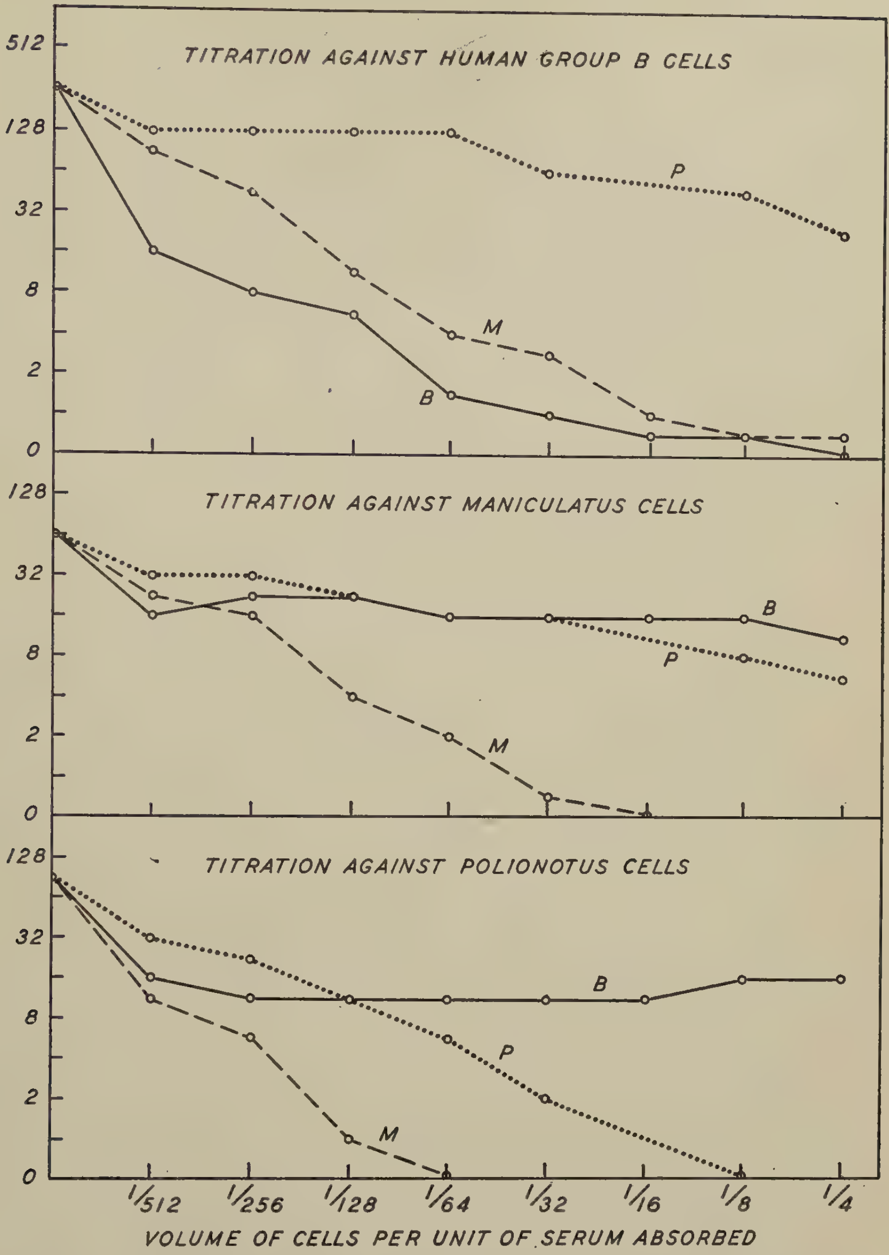

FIG. 1. Titration of serum A–121 against cells of *Peromyscus maniculatus* (*M*), *P. polionotus* (*P*), and human group B (*B*), after absorption with cells of the same three bloods.

latus, thus adding *polionotus* to the group of species suspected of possessing a B-like antigen. Whether the cells of *maniculatus* and *polionotus* are capable of completely absorbing the β agglutinin of group-A sera when used in larger quantities has not been ascertained.

Figure 1 further shows that human group-B cells were able to remove only a fraction of the anti-*Peromyscus* agglutinins, reducing the titer of the diluted A serum from about 64 to 16, after which excess B cells appeared to have no further effect. Such agglutinins, which remain in the human serum after exhaustion with human cells of homologous blood group, are termed heteroagglutinins and will be described further in the next section. Both anti-*maniculatus* and anti-*polionotus* agglutinins were removed more rapidly by *maniculatus* than by *polionotus* cells. According to this single series of tests, a group-A serum having an anti-*maniculatus* titer of 64 should provide the most satisfactory reagent for differentiating *maniculatus* and *polionotus* after absorption with one-fourth volume of *polionotus* cells. Titration curves similar to those of Figure 1 were obtained in tests with two other group-A sera.

TESTS WITH PURIFIED ISOAGGLUTININ β AND WITH HETEROAGGLUTININS OF GROUP-A SERA

The presence of a B-like antigen in *Peromyscus* cells of species *maniculatus,* F_1 *maniculatus* × *polionotus* hybrids, *polionotus,* and *leucopus* has been demonstrated by the capacity of these cells to reduce the titer of group-A sera against human B cells. A more decisive method for the detection of such substances, however, is considered to be furnished by the use of "purified" solutions of the isoagglutinin β. These are prepared by the process of "splitting off" the agglutinin bound to group-B cells that have been agglutinated by group-A serum. This method takes advantage of the fact that the isoagglutinins are released from the antigen-antibody complex by the application of heat. Three such solutions were prepared from different group-A sera, following the technique of Landsteiner and Miller (1925). Serum A–121 yielded the most concentrated solution, and the results of its titration against a small series of *Peromyscus* bloods are given in Table III. In addition to human cells of groups B and O, suspensions of two beef bloods were included as controls, since it is known that cattle possess a B-like antigen. Although the titers were smaller, the reactions of the two agglutinin solutions prepared from other sera were similar to those shown in Table III. Cells of *leucopus, gossypinus, maniculatus,* F_1 *maniculatus* × *polionotus* hybrids, and *polionotus* were all agglutinated about as strongly as human B cells, whereas cells of *nasutus, truei, eremicus,* and *crinitus* gave no trace of agglutination. The difference in reaction with untreated A sera in these two groups of species is thus shown to be due in large part to the presence or absence of a B-like antigen.

Exhaustive treatment of group-A sera with group-B cells separates the β isoagglutinin from other agglutinins which may activate the cells of other animals. Such "heteroagglutinins" must be responsible for the agglutination of the species *nasutus, truei, eremicus,* and *crinitus,* since these species are apparently devoid of the B factor. Likewise, the cells of *leucopus, gossypinus, maniculatus,* F_1 *maniculatus* × *polionotus* hybrids, and *polionotus* must possess some heteroagglutinogens, as is evidenced by the inability of group-B cells to exhaust group-A sera of their agglutinins against these species. Furthermore, the tests described in Tables I and II and Figure 1 suggest that the various *Peromyscus* species may vary in their capacity to absorb the heteroagglutinin fraction of group-A sera. A number of experiments were therefore performed to investigate this variation quantitatively.

Figure 2 illustrates the differences in absorptive capacity of nine different *Peromyscus* bloods against the heteroagglutinins of serum A–121. This serum, diluted 1:4, was twice absorbed with equal volumes of group-B cells in order to remove the β agglutinin. Parts of this solution were then treated with cells of the nine mice, using five different volumes of each blood. Single blood samples, rather than pools of several bloods of the same species, were used for the absorptions. The resulting solutions were then titrated against cells of a single *P. maniculatus blandus* mouse. Owing to these restrictions on the quantities of absorbed serum and test cells, it was not possible to test the preparations against cells of more than one species. If the results depicted in Figure 1 are typical, however, then it might be expected that similar sets of titration curves would have been secured with test cells of other species. A stock of *P. maniculatus blandus* was adopted as a standard source of test cells because of the high sensitivity of *maniculatus* cells as indicated by previous tests. Two other experiments, using other B-exhausted group-A sera absorbed with various *Peromyscus* bloods, gave results similar

TABLE III

AGGLUTINATION REACTIONS WITH PURIFIED ISOAGGLUTININ β

Cells	Dilution of β Agglutinin Solution							
	2^0	2^{-1}	2^{-2}	2^{-3}	2^{-4}	2^{-5}	2^{-6}	2^{-7}
Human group-B	++++	++++	+++	++	++	+	±	−
Beef (1)	++++	+++	++	+	±	−	−	−
Beef (2)	+++	+	±	±	−	−	−	−
leucopus	++++	+++	+++	+	+	±	±	−
gossypinus	++++	++++	+++	++	+	±	±	−
maniculatus (1)	++++	++++	+++	++	+	±	−	−
maniculatus (2)	++++	+++	++	+	±	−	−	−
maniculatus (3)	++++	++++	+++	++	+	±	−	−
F_1 *maniculatus* × *polionotus* hybrids	++++	++++	+++	++	+	±	±	−
polionotus	++++	++++	+++	++	±	−	−	−
nasutus	−	−	−	−	−	−	−	−
truei	−	−	−	−	−	−	−	−
eremicus	−	−	−	−	−	−	−	−
crinitus	−	−	−	−	−	−	−	−
Human group-O	−	−	−	−	−	−	−	−

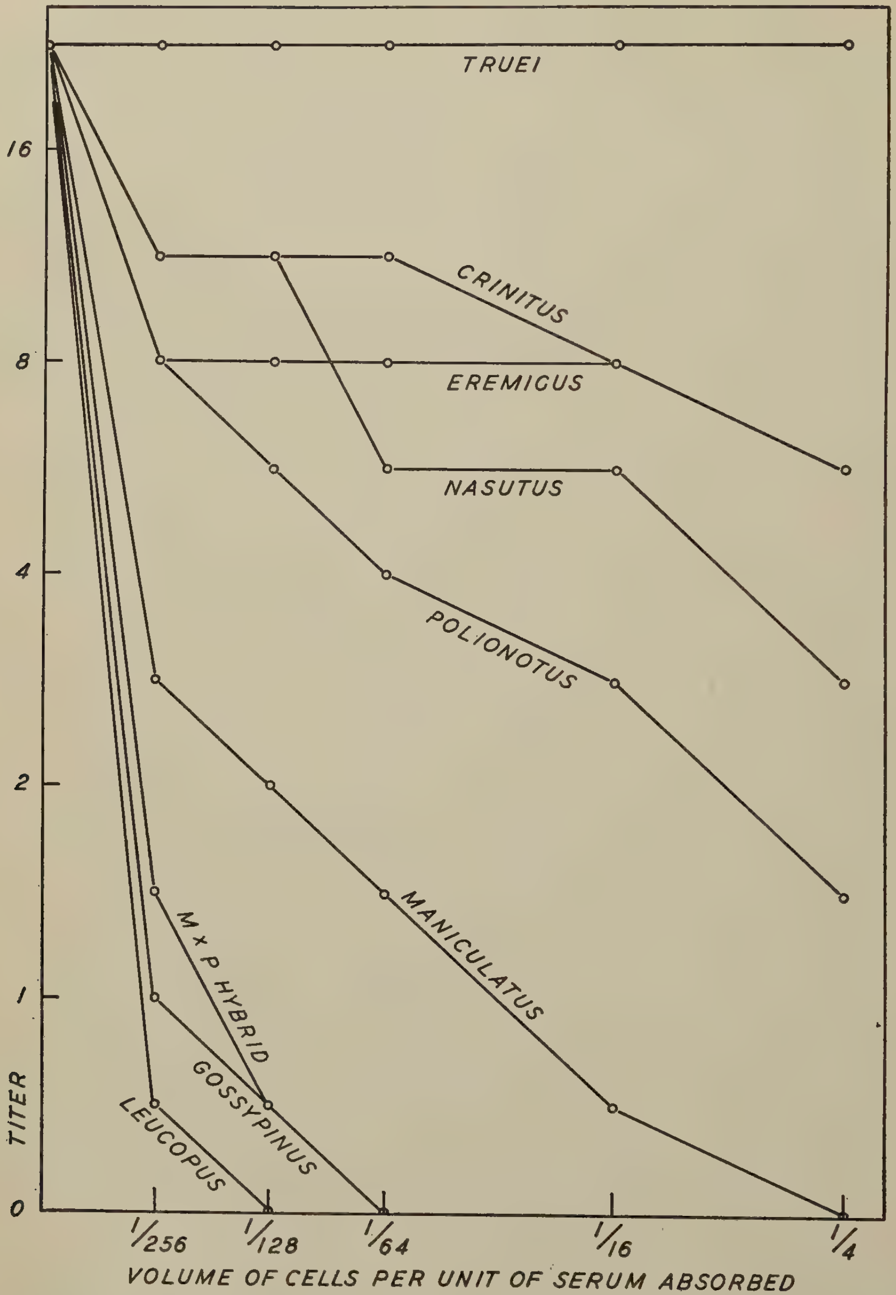

FIG. 2. Titration of the heteroagglutinin fraction of serum A-121 against cells of *Peromyscus maniculatus blandus*, after absorption with cells of various *Peromyscus* species.

to those of Figure 2. With the possible exception of *truei* and *nasutus,* the titration curves show species similarities which conform with the accepted taxonomic groupings.

DISCUSSION

B-like agglutinogens, reactive with human β agglutinin but not with anti-B agglutinins of immune rabbit sera, were regularly demonstrated by Landsteiner and Miller (1925) in New World monkeys (various species of *Cebus, Lagothrix, Mycetes, Chrysothrix,* and *Nyctipithecus*), as well as in the rabbit, guinea-pig, rat, mouse, cat, dog, cattle, donkey, and pig (von Dungern and Hirschfeld [1911], Landsteiner and Miller). Their presence has not thus far been observed in Old World monkeys (various species of *Papio, Cercopithecus, Cercocebus,* and *Macacus*), nor in the sheep, horse, or goat. Apparently, B-like antigens have been found in all rabbits and rodents thus far examined, with the exception of the four species of *Peromyscus* here reported. In contrast to the B agglutinogen of certain men, orangutans, and gibbons, the B-like antigens of the lower mammals appear to define specific differences rather than individual differences. It is of interest that the four *Peromyscus* species possessing the B factor are contained in the subgenus *Peromyscus,* whereas those lacking the factor belong to one cenospecies of the subgenus *Peromyscus* as well as to the subgenus *Haplomylomys.* Geographically, the B-negative group is confined largely to the southwestern part of North America. Unfortunately, there is no immediate prospect of investigating the genetics of this difference, since hybridization has not been observed between species belonging to these two groups (Dice, 1940).

For the genetical study of antigenic differences which are detectable with human heteroagglutinins, the two closely related species, *maniculatus* and *polionotus,* appear to offer the most promising material. A group-A serum exhausted of its anti-*polionotus* agglutinins still agglutinates cells of F_1 *maniculatus* × *polionotus* hybrids as well as cells of *maniculatus* (Table II). This might be explained by assuming that *maniculatus* mice possess one or more antigenic factors not present in *polionotus.* The presence of these factors in the hybrids would then be anticipated in accordance with a general rule of immunogenetics, namely, that genes determining the presence of antigens are dominant to those determining the absence of such antigens (or the presence of weaker antigens). In this connection, an agglutinogen reported by Gorer (1936) in certain strains of the house mouse is of interest. This property, which was demonstrable with human group-A sera (presumably with the heteroagglutinin fractions thereof), was shown to be determined by a single dominant gene. In Figure 2, the capacity of cells of the F_1 *maniculatus* × *polionotus* hybrid to absorb the heteroagglutinins against *maniculatus* cells is shown to exceed that of either parent species. This result has been observed in other tests and might suggest the inheritance of antigens from both species. The comparison of *blandus* and F_1 *maniculatus* × *polionotus* mice, however, is not entirely appropriate, since the former comprises a single subspecies of *maniculatus,* whereas the F_1 hybrids are offspring of *maniculatus* mice which are themselves hybrids of several subspecies. It is possible that these factors may show intraspecific variation, and the numerous subspecies of *maniculatus* and *polionotus* offer abundant material for further study.

It is of particular interest that the four *Peromyscus* species which react with the β agglutinin of group-A sera are also the four species which react most strongly with heteroagglutinins of the same sera (compare Table III and Figure 2). This correlation, which also appears to extend to variations within the B-positive group, suggests that the two agglutination systems may be chemically and genetically related. For example, it is conceivable that the same gene or genes may be responsible for major variations in both types of *Peromyscus* antigens. If so, the antigens reacting with at least some of the heteroagglutinins might be considered as special B components not contained in the human B complex. Friedenreich and With's (1933) interpretation of the human β agglutinin as a complex of several substances, some reactive only with human cells and some reactive with both human and animal cells, would thus be extended to include components reactive only with certain animal bloods. Further justification for this concept would be furnished if the heteroagglutinins of group-B sera should reveal antigenic differences between *Peromyscus* species unlike those demonstrated with group-A heteroagglutinins. Such comparative studies with human sera of groups B, O, and AB have not yet been made.

The two group-B sera (B–41 and B–518) that failed to agglutinate *Peromyscus* bloods, may also be of significance in relation to this problem. These two sera, which were provided by A. S. Wiener, were both obtained from group-B individuals who, by mistake, had received transfusions of group-A blood. The high isoagglutinin titers of these sera were therefore presumed to be due to the presence of immune α agglutinins in addition to the natural α agglutinins. The two sera were supplied in a dilution of 1 : 4, which may account for their negative reactions with *Peromyscus* cells. Even so, the heteroagglutinins against *Peromyscus* cells would be comparatively weak. It is therefore tempting to speculate that the formation of immune α antibodies may have resulted in a diminution of the animal heteroagglutinins in these sera.

Because of the interesting variations which have been observed in A and B substances of the body tissues and secretions of man and other animals, the testing of *Peromyscus* tissues is recommended for future study.

SUMMARY

Red blood cells of four species of North American mice, *Peromyscus leucopus, P. gossypinus, P. maniculatus,* and *P. polionotus,* possess an anti-

gen similar to the human isoagglutinogen B, whereas the cells of four other more distantly related species, *P. nasutus, P. truei, P. eremicus,* and *P. crinitus,* apparently lack this factor. The B-like antigen of *Peromyscus* is sufficiently similar to human B that it is activated by purified solutions of the human isoagglutinin β. On the other hand, like the B factors of other subprimate mammals, it is not reactive with anti-human-B agglutinins of immune rabbit sera.

Human group-A sera also possess other agglutinins (heteroagglutinins) against which the cells of various *Peromyscus* species show quantitatively different reactions. Although the numbers of mice tested are small, the variability within species appears to be considerably less than that between species. It is therefore possible to differentiate cells of *maniculatus* and *polionotus* by means of group-A sera which have been absorbed by cells of *polionotus.* The absorptive capacity of the cells of the F_1 hybrids of these two species appears to at least equal that of the stronger parent, *maniculatus,* suggesting the inheritance of dominant factors from this species.

The reactivity of different *Peromyscus* species to the β agglutinin appears to be correlated with the reactivity against heteroagglutinins of the same sera. This suggests that the two systems of agglutination may bear chemical and genetical relationship. In both serological properties the similarities between *Peromyscus* species are in general consistent with taxonomic groupings within the genus.

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VARIATION OF THE DEER-MOUSE (*PEROMYSCUS MANICULATUS*) IN A MOUNTAINOUS AREA OF SOUTHERN NEW MEXICO

BY LEE R. DICE

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INTRODUCTION

This study is of importance chiefly in showing the variability in several dimensions of the body and skeleton and in pelage color of a single subspecies of deer-mouse (*Peromyscus maniculatus rufinus*) within a small geographical area having a moderate amount of diversity in habitat types. The area studied lies in the mountains of Lincoln and Otero counties, southern New Mexico.

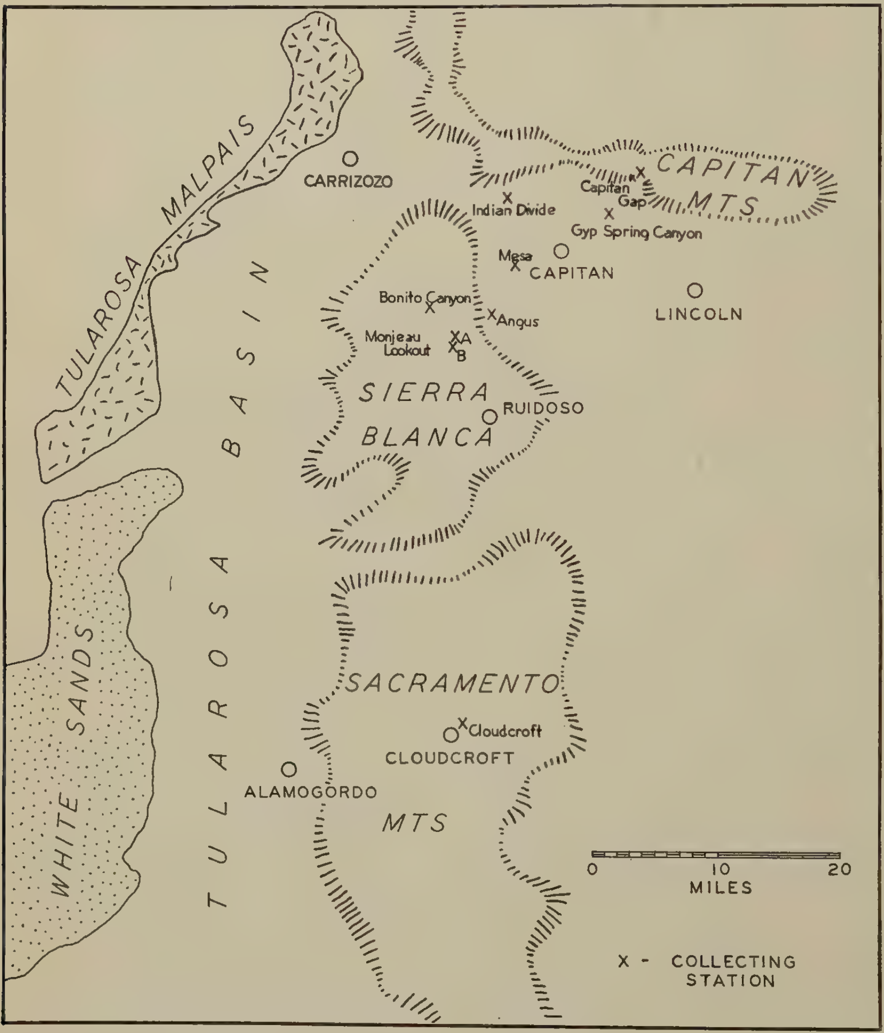
ACKNOWLEDGMENTS

The Cloudcroft stock was captured by the University of Michigan–Walker–Harris expedition of 1927 sent out by the Museum of Zoology. The Bonito Canyon stock was secured in 1928 by G. W. Bradt while on an expedition supported in part by the William P. Harris, Jr., Research Fund of the Museum of Zoology. The other stocks were secured by me in 1937 in connection with a field study supported in part by the Carnegie Institution of Washington. Most of the statistical calculations were made by Donald M. Clarke.

METHODS OF STUDY

The methods of study employed are the same as in my previous investigations of variation in *Peromyscus* (Dice, 1932). All of the animals here described were born in the laboratory and reared under nearly uniform conditions. All are of the one-year age class, and when killed for measurement were between the ages of thirty-six and seventy-eight weeks.

In the statistical comparisons between the stocks any difference between means greater than about 2.7 times its standard error has been considered to be significant. In the comparisons I have sometimes utilized also the standard deviations, making use of the fact that a difference between two means greater than the sum of their respective standard deviations indicates an overlap in measurements by less than 16 per cent of the individuals of the two populations concerned (Hubbs and Perlmutter, 1942).



MAP 1. Collecting localities in parts of Lincoln and Otero counties, southern New Mexico.

COLLECTING STATIONS

Most of the stocks of deer-mice here described were collected by me during the summer of 1937 (Dice, 1942). The stock from Cloudcroft was, however, collected in 1927 and that from Bonito Canyon in 1928. Measurements of the Bonito Canyon and Cloudcroft stocks have previously been published (Dice, 1933*a*). The stocks were secured at the following stations:

Angus. One and one-half miles northwest of Angus and nine miles in a direct line north of Ruidoso. Taken in yellow pine association at 7000 feet elevation.

Bonito Canyon. In the canyon of the Rio Bonito, twelve miles northwest of Ruidoso. Taken in meadows that were probably farm clearings, between 8000 and 9000 feet. The locality is now covered by the impounded waters above Bonito Dam.

Capitan Gap. On the south side of Capitan Gap, seven to eight miles in a direct line northeast of Capitan. In pinyon-juniper and yellow pine associations at 7000 to 7500 feet.

Cloudcroft. Within a mile of Cloudcroft. Taken mostly in Douglas fir association above 8000 feet.

Gyp Spring Canyon. Near Gyp Spring Canyon, four miles in a direct line northeast of Capitan. In short-grass association at 6200 feet.

Indian Divide. Just east of Indian Divide and six miles northwest of Capitan. In pinyon-juniper association at 7000 feet.

Mesa. Five miles southwest of Capitan. In pinyon-juniper association at 7000 feet.

Monjeau Lookout A. One-half mile southeast of Monjeau Lookout and seven miles northwest of Ruidoso. In oak brush association on a ridge at 9200 feet.

Monjeau Lookout B. One mile southeast of Monjeau Lookout and six and one-half miles northwest of Ruidoso. In yellow pine association at 8900 feet.

All these collecting stations are in Lincoln County, New Mexico, except the Cloudcroft station, which is in Otero County.

TABLE I

COLOR OF SURFACE SOILS FROM NEW MEXICO

Mean of ten tint photometer readings of each soil sample, in per cent

Station	Red	Green	Blue-violet
Angus	17.6	12.1	9.6
Capitan Gap	12.5	9.5	7.6
Gyp Spring Canyon	15.8	11.5	9.1
Indian Divide	20.4	14.8	12.5
Mesa	13.7	10.2	8.2
Monjeau Lookout A	18.3	12.4	10.6
Monjeau Lookout B	13.4	9.8	7.7

SOIL COLORS

The colors of the surface soils have been measured from a single sample taken at each of the collecting stations (Table I). No soil samples have been available from the Cloudcroft nor Bonito Canyon stations.

TABLE II

MEASUREMENTS OF *Peromyscus maniculatus rufinus* FROM NEW MEXICO
Means, standard errors, and standard deviations for one-year age class, both sexes, in millimeters

Stock	Number	Mean Age in Weeks	No. of Parents	Body Length	Tail Length	Foot Length	Ear Length
Angus	60	48	10	96.68 ± .48 (3.77)	64.61 ± .53 (4.16)	20.763 ± .081 (.646)	18.964 ± .073 (.580)
Bonito Canyon	75	44	13	92.40 ± .45 (3.87)	66.55 ± .52 (4.51)	20.175 ± .087 (.746)	18.032 ± .091 (.789)
Capitan Gap	98	53	12	97.24 ± .47 (4.67)	63.16 ± .35 (3.44)	20.217 ± .063 (.623)	18.406 ± .083 (.824)
Cloudcroft	128	47	32	86.60 ± .37 (4.17)	70.25 ± .45 (4.98)	20.171 ± .065 (.736)	18.376 ± .070 (.782)
Gyp Spring Canyon	115	57	14	98.41 ± .41 (4.43)	66.42 ± .41 (4.42)	20.444 ± .061 (.656)	18.569 ± .067 (.710)
Indian Divide	60	55	12	95.28 ± .55 (4.23)	62.90 ± .49 (3.72)	20.377 ± .097 (.751)	18.502 ± .094 (.700)
Mesa	54	49	11	95.48 ± .45 (3.29)	66.80 ± .48 (3.49)	20.433 ± .074 (.547)	18.635 ± .086 (.632)
Monjeau Lookout A	77	56	12	96.99 ± .47 (4.12)	63.08 ± .44 (3.87)	20.155 ± .108 (.947)	18.514 ± .084 (.719)
Monjeau Lookout B	81	53	15	96.16 ± .48 (4.29)	63.54 ± .40 (3.55)	20.096 ± .074 (.664)	18.267 ± .087 (.783)

Stock	Femur	Mandible	Condyle-premaxilla	Condyle-zygoma	Bullar Width
Angus	16.268 ± .083 (.635)	16.217 ± .052 (.397)	24.659 ± .074 (.567)	17.592 ± .053 (.405)	10.932 ± .035 (.268)
Bonito Canyon	16.005 ± .070 (.553)	16.054 ± .052 (.431)	24.328 ± .079 (.639)	17.258 ± .059 (.474)	10.794 ± .034 (.276)
Capitan Gap	16.480 ± .064 (.615)	16.341 ± .040 (.385)	24.678 ± .062 (.604)	17.685 ± .041 (.399)	10.982 ± .034 (.330)
Cloudcroft	15.728 ± .064 (.693)	16.087 ± .041 (.442)	24.403 ± .065 (.671)	17.298 ± .041 (.430)	10.784 ± .024 (.255)
Gyp Spring Canyon	16.704 ± .069 (.733)	16.450 ± .044 (.471)	24.914 ± .070 (.740)	17.788 ± .044 (.465)	10.978 ± .030 (.318)
Indian Divide	16.130 ± .107 (.790)	16.116 ± .061 (.452)	24.275 ± .094 (.704)	17.298 ± .073 (.544)	10.822 ± .040 (.298)
Mesa	15.927 ± .077 (.557)	16.346 ± .067 (.486)	24.585 ± .080 (.577)	17.588 ± .057 (.413)	10.958 ± .027 (.194)
Monjeau Lookout A	16.216 ± .092 (.803)	16.370 ± .055 (.483)	24.707 ± .084 (.734)	17.643 ± .062 (.540)	10.958 ± .034 (.297)
Monjeau Lookout B	16.031 ± .061 (.531)	16.152 ± .052 (.459)	24.372 ± .071 (.632)	17.454 ± .055 (.491)	10.773 ± .030 (.269)

The differences in surface soil color between the several stations are moderate in amount. The soil at none of the collecting localities is either exceptionally dark or exceptionally pale.

VARIATION IN DIMENSIONS

Each of the stocks here described exhibits a moderate amount of variability in the dimensions of the body and of the skeleton, as measured by the magnitude of their standard deviations (Table II).

None of the stocks are consistently either very large or very small in their dimensions when compared with the other stocks from this region. The means of the several measurements differ between the stocks, and certain of the differences are of high statistical significance when evaluated by their standard errors. The magnitude of the differences in means is, however, usually not very great. The difference in mean condylo-premaxillary skull length between the Gyp Spring Canyon and Indian Divide stocks, for instance, is 0.639 ± .117 millimeter. This is one of the largest differences in skeletal measurements. In body length the Cloudcroft stock averages small, and its mean is exceeded by the means of all the other stocks, except the one from Bonito Canyon, by amounts greater than the sums of the two respective standard deviations. The difference in mean body lengths between the Gyp Spring Canyon stock, which is the largest in this dimension, and the Cloudcroft stock is 11.81 ± .55 millimeters. The sum of the two standard deviations is 8.60 millimeters. None of the other differences between the stocks in the mean measurements of either the body or the skeleton are as great as the sum of the two respective standard deviations.

VARIATION IN PELAGE COLOR

A moderate amount of variation in pelage color is present in nearly every one of these laboratory-bred stocks. The usual range of variation is from a dull buff to a dull gray. In the Capitan Gap stock a few specimens are bright buff and a few others gray, similar, respectively, to the buff and gray color phases of *blandus* (Dice, 1933*b*). Most of the Capitan Gap specimens, however, are intermediate in hue between buff and gray, and no separation of the stock into buff and gray phases is possible.

The palest pelage colors among the mice here described are exhibited by the stocks from Gyp Spring Canyon and Capitan Gap (Table III). These stocks rank high in tint photometer readings both for the dorsal stripe and the side of the body. The Gyp Spring Canyon stock is significantly exceeded by the Capitan Gap stock only in readings for dorsal stripe blue-violet.

The Indian Divide stock ranks high in tint photometer readings for dorsal stripe red and green, but in the other color readings it is intermediate among the stocks. The Mesa, Angus, and Monjeau Lookout A stocks rank intermediate among the stocks in all the color readings.

The Cloudcroft stock has significantly lower tint photometer readings for the dorsal stripe than has any other stock from this region. It ranks low also in the readings for side green and side blue-violet, but it significantly exceeds the Monjeau Lookout B stock in readings for side red.

The Monjeau Lookout B stock has significantly lower readings for side red than has any other stock. Its readings for side green and side blue-violet also rank low. In the readings of the dorsal stripe, however, it has significantly higher readings than has the Cloudcroft stock.

The Bonito Canyon mice also have relatively dark pelage colors. For the readings of the dorsal stripe they significantly exceed only the Cloudcroft mice; in the readings for side green and side blue-violet they significantly exceed only the Cloudcroft and Monjeau Lookout B mice; but their readings for side red are high, for these average significantly lower only than those of the Gyp Spring Canyon mice.

TABLE III

PELAGE COLOR OF *Peromyscus maniculatus rufinus* FROM NEW MEXICO
Mean tint photometer readings, standard errors, and standard deviations for one-year age class, both sexes, in per cent

Stock	Dorsal Stripe		
	Red	Green	Blue-violet
Angus	10.00 ± .16 (1.28)	7.34 ± .12 (0.96)	5.23 ± .10 (0.77)
Bonito Canyon	9.29 ± .21 (1.66)	5.97 ± .17 (1.34)	4.66 ± .12 (1.00)
Capitan Gap	11.57 ± .20 (1.90)	8.77 ± .17 (1.53)	6.93 ± .14 (1.29)
Cloudcroft	7.90 ± .11 (1.29)	5.12 ± .06 (0.76)	4.01 ± .05 (0.61)
Gyp Spring Canyon	11.54 ± .16 (1.62)	8.53 ± .11 (1.10)	6.38 ± .09 (0.94)
Indian Divide	11.40 ± .28 (2.13)	8.48 ± .19 (1.49)	6.20 ± .16 (1.20)
Mesa	10.42 ± .17 (1.33)	8.02 ± .13 (1.00)	6.29 ± .14 (1.04)
Monjeau Lookout A	9.99 ± .16 (1.38)	7.68 ± .13 (1.13)	6.01 ± .10 (0.91)
Monjeau Lookout B	8.62 ± .17 (1.48)	6.42 ± .14 (1.20)	4.81 ± .10 (0.90)
	Side Stripe		
Angus	18.70 ± .24 (1.89)	13.36 ± .20 (1.58)	9.67 ± .14 (1.16)
Bonito Canyon	20.41 ± .30 (2.38)	12.92 ± .21 (1.66)	9.58 ± .20 (1.60)
Capitan Gap	20.93 ± .25 (2.32)	15.43 ± .19 (1.79)	11.84 ± .17 (1.53)
Cloudcroft	19.50 ± .19 (2.24)	12.14 ± .12 (1.48)	8.83 ± .10 (1.15)
Gyp Spring Canyon	21.74 ± .27 (2.67)	15.80 ± .18 (1.79)	11.44 ± .14 (1.38)
Indian Divide	20.20 ± .34 (2.59)	14.60 ± .23 (1.78)	10.72 ± .19 (1.43)
Mesa	19.81 ± .28 (2.12)	14.48 ± .21 (1.62)	10.78 ± .18 (1.40)
Monjeau Lookout A	19.13 ± .25 (2.23)	14.08 ± .20 (1.81)	10.65 ± .18 (1.57)
Monjeau Lookout B	15.99 ± .33 (2.94)	11.49 ± .25 (2.19)	8.38 ± .18 (1.63)

When comparisons between these stocks are evaluated by means of the standard deviations it is evident that the means of the tint photometer readings of the dorsal stripe for all three color screens are lower for the Cloudcroft stock than are those of the stocks from Capitan Gap, Gyp Spring Canyon, and Indian Divide. The difference in each comparison is greater than the sum of the two respective standard deviations. For dorsal stripe green and blue-violet the differences between the Cloudcroft and the Mesa and Monjeau Lookout A means also are greater than the sums of the two respective standard deviations. For dorsal stripe green, furthermore, the Cloudcroft mean is less than the Angus mean by an amount that is greater than the sum of the two respective standard deviations.

In tint photometer readings of the side stripe the mean of the Gyp Spring Canyon stock for red is higher than that of the Monjeau Lookout B stock by an amount greater than the sum of the two respective standard deviations. In readings for side stripe green the Gyp Spring Canyon stock exceeds the Cloudcroft and Monjeau Lookout B stocks, and the Capitan Gap stock exceeds the Cloudcroft stock, by amounts greater than the sums of the respective standard deviations. In readings for side blue-violet the Capitan Gap and Gyp Spring Canyon stocks both exceed the Cloudcroft and Monjeau Lookout B stocks by amounts greater than the sums of the respective standard deviations. No other differences in color readings as great as this are exhibited by any other of the stocks.

It is evident therefore that of the stocks here compared the palest in pelage are those from Gyp Spring Canyon and Capitan Gap. The Cloudcroft and Monjeau Lookout B stocks have the darkest colors. Some of the differences between the stocks are of considerable statistical significance, though none are very great in amount. The difference in mean tint photometer readings for dorsal stripe red between the Capitan Gap and Cloudcroft stocks is 3.67 ± .23 units. The mean difference for side stripe red readings between the Gyp Spring Canyon and Monjeau Lookout B stocks is 5.75 ± .43 units. These are the greatest differences between any of the stocks.

DISCUSSION

The statistical comparisons that have been made in this report apply, of course, only to the laboratory-bred stocks and not to the wild populations living at the localities where the parents of the laboratory stocks were collected (Hovanitz, 1942: 179–81). Any group of individuals captured at a particular station which becomes the parents of a laboratory stock will carry an inadequate sample of the genes of the whole wild population. There is no way of estimating how adequate each sample is. Nevertheless, the measurements of the laboratory stocks do give an indication of the characters of the wild populations and of their variabilities. Series of *Peromyscus* taken in the field have shown general agreement in measurements with series bred in the laboratory (Dice, 1933*a:* 5–7; 1937: 10–17).

The collecting stations for the deer-mice described in this report are in the oak-brush, yellow pine, and Douglas fir associations of the montane belt and in the short-grass and pinyon-juniper associations of the sabinal belt (Dice, 1930 and 1942). The ecologic diversity between these stations is only moderate in amount. The patches of oak brush and of short grass in these belts are generally small in size, and most of the area of both the montane and sabinal belts is covered by more or less dense woodland or conifer forest. Elevations range from 6200 feet at Gyp Spring Canyon to 9200 feet at the Monjeau Lookout A station.

All the stations except the one at Cloudcroft lie within an area about twenty-five miles in greatest diameter. The Cloudcroft station lies about twenty-five miles farther south. None of the stations therefore are distant in a direct line from one another more than about fifty miles.

Within this relatively small area of moderate diversity in habitat types it is not surprising that no great variation in the body dimensions of the deer-mice is exhibited. The small differences in the dimensions of the body and its parts that do distinguish these several stocks may be in part due to errors in the sampling methods employed. It is probable, however, that there are some differences between the various local populations in the aggregate of genes that affect body size. There is no evidence that the differences in body dimensions between the several stocks are correlated in any way with differences between the types of environments at the several collecting stations.

The pelage color, as measured by the tint photometer, differs considerably among the stocks. Some of the differences between the stocks in pelage color might be expected to be correlated with differences in the heaviness of the vegetation and with the surface soil color at the collecting stations. Such correlations are known to exist in other regions (Dice and Blossom, 1937: 92–108).

A tendency toward a correlation between the darkness of pelage of the deer-mice and the heaviness of the vegetation at their collecting stations is especially suggested by certain of the stocks. The Cloudcroft and Monjeau Lookout B stocks have on the average the darkest pelage, and these stocks came from the heaviest conifer forests in the area. Conversely, the Gyp Spring Canyon stock, which averages pale in pelage, is from open short-grass association. The Capitan Gap stock, which also averages pale in pelage, came from moderately heavy pinyon-juniper and yellow pine forest. It should be pointed out, however, that the Capitan Gap and Gyp Spring Canyon localities lie immediately adjacent to one another and that the two collecting stations were less than three miles apart. The general rule that the pelage color of these mice tends to be dark in heavy forest and pale in open situations, seems therefore to be here modified locally by the influence of an adjacent community of contrasting ecologic type.

No close correlation between pelage color and soil color is evident among these stocks. It is true that the soil color at the Monjeau Lookout B station is relatively dark (Table I) and that the pelage color of the mice likewise averages dark. However, the soil at the Capitan Gap station is also dark, and the mice of that stock average pale in pelage. Further, the Gyp Spring Canyon mice average pale in pelage, and the soil at their collecting station ranks intermediate.

When considering the general tendency toward a correlation between the pelage color of the deer-mice and the heaviness of the vegetation and the apparent absence of a correlation between pelage color and surface soil color, it should be borne in mind that in this small region there is a considerable variety of vegetation, but no strong variation in soil color. Furthermore, it must be remembered that all these collecting stations are fairly close together. There are no evident barriers here to the movements of the deer-mice, and these highly mobile mammals undoubtedly at times move considerable distances. It would be expected, therefore, that there would be considerable interbreeding between the various local populations. This interbreeding would theoretically tend to prevent the production of strongly marked local races.

Nevertheless, the evidence from the laboratory-bred stocks indicates that some of the local wild populations differ from others in certain average characters. Some of the variations in pelage color among the stocks tend to be correlated with variations in the types of vegetation growing at the several collecting stations. Many of the differences between the stocks have, on the contrary, no obvious ecologic relationship and probably indicate only the presence of random local variations in the hereditary constitutions of the several wild populations. The precise amount of difference in genetic constitution between the various local wild populations cannot, however, be measured by the data here presented, because of the errors of sampling involved in the production of the laboratory-bred stocks.

SUMMARY

Measures of the body and skeletal dimensions and of pelage color are given for nine laboratory-bred stocks of deer-mice from a small area in the mountains of southern New Mexico, where there is a moderate amount of habitat variability. Some of the differences between the means of the several stocks are of high statistical probability, though none are very great in amount. There are greater differences between the stocks in pelage color than in body dimensions. Insofar as the laboratory-bred stocks represent the characters of the wild populations at the places where their parents were trapped, there is an indication that the depth of pelage color of the deer-mice tends to be correlated with the heaviness of vegetation in their habitats. No other ecologic trend is apparent. Some of the differences between the stocks are presumably due to random variations in genetic constitution between the local wild populations inhabiting the various parts of the area studied.

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SPECIATION IN LIMPETS OF THE GENUS *ACMAEA*

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INTRODUCTION

THE occurrence within a circumscribed area of several closely related but distinct species of organisms poses many difficult questions about their possible mode of origin. A particularly puzzling example of this kind is presented by the limpets of the genus *Acmaea.* Along the Pacific coast of North America, indeed within the limits of the state of California, there dwell no less than seventeen species of the genus *Acmaea* (A. R. Test, MS). Further, they all live within the very narrow vertical limits of the intertidal zone along the ocean shore. The problem of the mode of origin of this diverse group of species deserves intensive study.

Of the seventeen species living along the coast of California, eight range both north and south beyond the limits of the state, although not all their ranges extend the entire length of the west coast of North America. These species are *pelta* Eschscholtz, *digitalis* Eschscholtz, *asmi* (Middendorff), *paleacea* (Gould), *rosacea* Carpenter, *limatula* Carpenter, *scabra* (Gould), and *triangularis* Carpenter. Two others, *insessa* (Hinds) and *mitra* Eschscholtz, have been accorded very lengthy ranges, but I have not been able to substantiate these ranges from the collections I have seen. The ranges of the seven remaining species terminate within the state of California, but extend beyond one border, either to the north or the south. The species ranging north of the state of California are *testudinalis scutum* Eschscholtz, *persona* Eschscholtz, *depicta* (Hinds), *instabilis* (Gould), *fenestrata cribraria* (Gould), and *ochracea* Dall; *conus* Test (1945 *a* and *b*) ranges south of California.

There is actually a brief area along the coast of California, extending from Point Concepcion south to Point Fermin (San Pedro), where all seventeen of these species are present. When the narrow limits of the intertidal zone are taken into consideration, this is remarkable and very difficult to explain on the basis of complete geographic isolation for every species at some previous time in order to permit speciation to develop.

These species came into existence in this limited area in part because of the extremely limited movements of each animal during its lifetime, in part because of the relative rigidity of the microhabitat limitations of each species. These two factors have permitted speciation to take place by geographic isolation of the usual type and also by ecologic segregation of an unusual type, in which isolation may be a matter of only inches, or at most a foot.

Although I worked with the group originally only from the taxonomic point of view, I became interested in the problem of the origin of several species in a limited area. As my studies of the natural history of the group proceeded (A. R. Test, 1945*c*), ideas suggested how such a situation came to exist, and it seems worthwhile to present them here, as pertaining not only to the group under consideration, but perhaps also to other groups.

The study of which this paper records the results was carried out under the guidance and with the encouragement of Professor S. F. Light at the University of California, to whom I wish to express my very great gratitude.

The specific nomenclature used in this paper follows that in a manuscript which will be published under the title, ''The Taxonomy, Natural History, Phylogeny, and Bibliography of the Genus *Acmaea*.''

TERMINOLOGY

In order that there will be no misunderstanding as to what is meant in this paper by certain terms which have been used differently by previous authors, I define my usage for each term.

SPECIES is here understood to mean a morphologically distinguishable and interbreeding group of animals which do not appear to interbreed with any other morphologically distinguishable group, since no intergrades have been found.

A SUBSPECIES, the only category for which a trinomial name will be used in this paper, is always a geographic race, as defined below. No other group of variants and no individual variants will be given a trinomial name. For the sake of clarity, variants which have received names in the previous literature are discussed and synonymized in the manuscript already cited.

SPECIATION is here understood to mean those processes during which divergence is established and reproductive isolation is accomplished so that a ''parent species'' breaks up into two or more daughter species (May, 1942: 23).

GEOGRAPHIC SPECIATION is that differentiation which has developed during a recognizable and measurable geographic isolation of the parent and offshoot stocks from each other, by the gradual accumulation of inherited differences which make morphological distinction and which prevent interbreeding.

ECOLOGIC SPECIATION is that differentiation which has taken place during isolation of extreme variants which could not survive in the parental microhabitat, but which are able to take over a microhabitat not compatible with the parental morphology and ecology. This isolation is not of a geographic type, but is due to the unsuitability for the parent stock of the microhabitat invaded by the offshoot, and to an intervening area, however small, which cannot be crossed by individuals of either stock, such as is provided by the holdfasts of some of the algae and by silt and sand surrounding the base of the rocks or plants occupied by the offshoot stock.

An ECOLOGIC VARIANT is a nonhereditary phenotype which differs from most members of the species in some definite, recognizable manner. For example, in bays or harbors, where the wave action is slight but the chemical contents of the water are heavily concentrated with industrial wastes or detritus from organic sources, individuals of all species represented have heavier than normal shells, humped-up shape, with reduced aperture, and increased ornamentation. Such variations predominate in all species wherever such areas occur.

INDIVIDUAL VARIANT refers to a phenotype produced by a particular combination of hereditary traits.

EURYTOPIC refers to a broad tolerance of diverse environmental conditions on the part of a species population. Such species are able to survive in many recognizably diverse situations, the microhabitat having only broadly recognizable limits.

STENOTOPIC refers to a rigidly restricted tolerance to variation in environmental conditions on the part of a species population. Such species are found only in one particular kind of microhabitat, always the same in all or most all of its recognizable features.

LIMITING CONDITIONS are those factors in the habitat to which the individuals must adapt or fail to survive.

DOMINANT SPECIES, FORMS, OR VARIANTS are those which are most abundant in the area or region under discussion.

FIELD STUDIES

Field studies were conducted along the coast of California, for a period of six years (1931 to 1937). Visits were made to every important, and many small, reefs, as well as to estuaries, sloughs, and river mouths, from the northern boundary of the state south to Point Fermin, San Pedro, California. Certain situations were visited repeatedly, the most frequently worked over being Moss Beach, San Mateo County; Monterey; Jenner, Sonoma County; and the reefs between and around Bolinas and Tomales Bay, Marin County. Special trips were made to work at and near Points Concepcion and Arguello, Santa Barbara County.

CRITERIA FOR SPECIES

For working out the relationships of the various species of limpets, I have not found the shell a reliable guide, probably because it bears the brunt of environmental contact. It is particularly susceptible to the development of adaptive characters and hence liable to both convergent and divergent evolution. The shell of a limpet is always in contact with the environment, is the part of the animal most likely to be affected by it, and, therefore, is least likely to indicate relationships accurately. Direct contact with the environment has resulted in numerous ecologic variants, many of which, under special conditions, have become dominant in given localities. These variants have added to the confusion in the relationships of the taxonomic forms.

The unreliability of the shell for showing taxonomic relationships is well illustrated by the fact that limpets in similar ecologic situations the world over have shells of practically the same general contour, size, weight, and often sculptural pattern. This parallelism is so general that on the basis of the shell alone it is frequently possible to determine the ecologic situation from which any given species or group of individuals may have come. It is hardly necessary to add that species geographically far removed from each other, although equivalently situated ecologically and possessing similar shell characters, are not likely to be closely related. Instead, a given species will be more closely related to another often very different in architectural pattern of shell but occupying the same or adjacent geographic areas than to species with architecturally equivalent shells but living in widely separated geographic ranges.

A slightly less significant illustration is provided in shell characters by predictable variations on the basis of ecologic influence. Some individuals of a species living under ecologic conditions different from the normal in some controlling factor or factors will often differ in certain characters from normal individuals in a particular manner. Such variations are predictable in connection with known variable ecologic factors which result in conditions below optimum for the species. Among the well-established species on the coast of California it is not uncommon to find the same architectural principles being utilized within several normally very different species by individuals subjected to the same kind of unfavorable environmental conditions, such as lowered salinity, increased chemical content of the water, or increased wave action.

Obviously, this tendency to vary with the ecological situation practically eliminates the shell as a factor in determining species relationships and necessitates the use of some other structure that is more dependable than the shell for defining these relationships. The structure must not be too plastic under ecologic influences, nor yet so completely removed from the environment as to prevent accumulation of small specific changes. The basal ribbon of the radula appears to be such a structure.

The radula, well enough protected from outside influences during its development, later regularly is in contact with a specific part of the environment, namely, the food and its substrate. This, significantly, is the least variable part of a limpet's environment, owing to the specific nature of the feeding habits of most limpets. Hence, there is little tendency for the radula to vary within species. In the past, use of the radula has been limited to a study of the teeth, which provide poor criteria for specific determination because of change due to injury and wear. I have used the basal ribbon of the radula, stripped of teeth; it offers a reliable basis for identification as well as for the determination of specific relationships, because it is not especially subject to selective action, injury, or wear and tear. The ribbons appear to have acquired specific characters chiefly because of the inevitable accumulation of small inherited differences.

As suggested above, the use made of the radula in the past has been open to criticism, due largely to the methods employed. Dall (1871) wisely based his subgenera of *Acmaea* upon radular characters, but unwisely used tooth shape and arrangement as the criteria for specific differentiation. Oliver (1926) made groupings of species, which he called genera, differentiated partly by tooth characters. The validity of his results was further weakened by the use of a combination of structural characters of the shell with radular characters for generic criteria. Since shell characters are not reliable indices of relationships, genera based upon them, even though in combination with radular characters, are of dubious value.

Because of these difficulties with a structure which should present ideal material for specific differentiation, J. A. Perino, of the University of California, several years ago worked out a method of preparing the radula to eliminate the weaknesses mentioned above, and to make use of the taxonomic possibilities of the structure. In a properly treated radula the teeth can easily be scraped off, the basal ribbon stained, and a thoroughly useful, specifically stable structure obtained which shows excellent specific differentiation. It is this structure, the basal ribbon, which I have used. A complete description of the preparation of the radular ribbons will appear in a subsequent paper (A. R. Test, MS).

An ambitious attempt was made to obtain length-to-width-to-height proportion ratios of the shells for the various species in order to use them in determining the specific limits, but the only result of the tedious measuring of approximately three thousand individuals of several species was the conclusion that size and proportion in limpets are too much influenced by ecologic irregularities to have any practical significance.

Each species which I recognize (A. R. Test, MS) has been diagnosed on the basis of criteria indicated as reliable by comparison of shell characters with radulae. Many of the species exhibit variability (genetic and /or somatic), either ecologic, individual, or geographic in character. In the past, this extensive variability has led to the subdivision of these species into many categories (so-called subspecies, varieties, forms). Most of these have no place in a nomenclatural system, since they are based upon differences which are not geographically segregated and seem to have no correlation with ecologic or physiologic segregation or tendencies thereto. Indeed, these categories often have been used for mere variants representing naturally occurring diversities in genetic combinations in a large heterozygous population, as well as for variants resulting from the adaptability of the individual to differing environmental conditions and to variations produced by extraneous causes (fungus infection, unfavorable environmental conditions, substrate limitations, and injuries).

Related species should manifest their relationship by any feature in inverse ratio to the amount of divergent adaptive evolution which that feature has undergone. Such is, indeed, the situation among the species of *Acmaea.* The extent of resemblance of the shells of related species depends not upon closeness of relationship, but rather upon whether or not the diverging species have remained in similar microhabitats. The radular ribbon on the other hand, less subject to adaptive evolution than is the shell, and hence not subject to the phenomena of rapid divergent or convergent evolution, indicates the actual relationship fairly accurately, regardless of whether the species in question have remained in equivalent microhabitats or have become adapted to differing situations.

METHODS OF SPECIATION

The importance of geographic subspeciation has long been understood, namely, that it seems frequently to lead to the origin of species. This appears to be the case when geographic subspecies are isolated from the main stock long enough for evolution to proceed to the point at which the isolated group can or will no longer interbreed with the main stock. Students of the genus *Acmaea* do not appear to have grasped this fact and have classed as subspecies any striking variant, regardless of its geographic coincidence with the main stock of the species, that is, with the other so-called subspecies. Every subspecies (geographic race) obviously must occupy a range which is geographically complementary to that of other subspecies of the same polytypic species (*rassenkreis* of Rensch, 1934), except within a limited area of intergradation.

To be sure, any individual phenotype common in one subspecies may appear as an occasional phenotype in any other subspecies of the same species, but each subspecies will be composed for the most part of phenotypes which appear only infrequently in any other subspecies. The bulk of the population of one subspecies differs from the bulk of the population of any other subspecies of the same species with regard to those phenotypes which predominate. Thus, a sample of any subspecific population may be recognizable by its proportions of phenotypes rather than by peculiar kinds of phenotypes. Effective isolation is then necessary over a sufficiently extended period to permit completion of the process of speciation.

Within the genus *Acmaea*, much speciation has apparently been due to the geographic process. This is indicated by the geographic ranges of some of those species which have been shown by studies of the radular ribbons to be closely related, as well as by those *Acmaea* polytypic species which are extant today.

Geographic speciation, has apparently not been the only kind to occur within the genus; indeed, it has probably been the less common type. A careful survey, including ecologic and relationship studies, of species of *Acmaea* occurring on the coast of California, has in certain instances failed to yield the slightest evidence in favor of geographic speciation. The information obtained has even suggested that such a method could not have been possible. For example, along the west coast of North America occur nine species of one subgenus (*Collisella*) affording every indication of close relationship, yet having either completely concurrent or encompassed ranges. Inasmuch as the obviously parental form has the greater range in every instance in which the range of one species is encompassed by that of another, and since the range and population midpoints of the species (Schenck and Keen, 1936) do not differ, it becomes practically impossible to explain the situation in terms of geographic speciation.

This study, however, has shown that in every example in which the related species have concurrent (or encompassed and encompassing) ranges the microhabitats are not equivalent. In fact, the microhabitats are so different that the various species are unable to invade each other's microhabitat and furthermore are isolated from one another by barriers as effective as a geographic separation, though very small in size. This isolation is emphasized by the relatively slight motility in animals of this genus.

Studies have indicated that the limpet moves during its lifetime at most within a circumscribed area of a few feet. Its limited movements are apparently for the purpose of finding food and are at rather infrequent intervals. Individuals of two species customarily return to their starting point at the end of one of their forays (Hewatt, 1940; A. R. Test, 1945*c*), whereas individuals of other species fail to do so (Richardson, 1934; Villee and Groody, 1940; F. H. Test, 1945). Whether or not a return to a home site is made, the distance traversed by any limpet within its lifetime is very limited, actually, to an area of a square foot or two at most. Consequently, there is great isolation of adults, and mating opportunities are largely limited to near-by individuals. There is further limitation of movement by substrate, since few individual limpets will leave a suitable subtrate to venture upon an unsuitable one, even in the search for a new feeding ground.

The presumably ancestral species in every instance is a species of greater variability and greater ecologic tolerance than the presumably descendant species, and the ancestral species includes among its variants some which approach the general structural lines of the descendant species. These facts provide the basis for an apparently satisfactory solution of the speciation problem in this group. They suggest that speciation in such cases has been caused by ecologic segregation of particular extreme variants of a variable species within a microhabitat different in its controlling factors from that of the parental species, and their effective isolation thereby. These variants are such as could not survive within the normal confines of the parental microhabitat, but are able to invade some other microhabitat unoccupied by and unavailable to less extreme members of the parental species. Released from the pressure of population and isolated from other variants of the parental species by sharply delimiting ecologic barriers and by the lack of motility, these variants should provide material for selective and geologically rapid development of a new form which could attain the qualifications of a new species.

Such a process as speciation by ecologic segregation depends, however, upon certain assumptions. Most important are those with regard to breeding habits. If speciation has proceeded by the gradual process of selection of mutations and extreme variations, complete segregation of the new variant from the parent stock must have been effective to permit final fixation of the characters of the new species. This would be the case if the mutations involved prevented cross breeding of the mutants and the parent stock, or if the type of fertilization required close approximation of male and female, although not necessarily internal fertilization.

Curiously enough, the literature is very contradictory with regard to the method of insemination in *Acmaea*. It is Willcox' opinion (1898: 444; 1905: 332–33) that, possibly, the limpet possesses an intromittent organ in the renal papilla, and that, probably, mantle cavity fertilization takes place. Her evidence is neither conclusive nor convincing, but her belief is mentioned for what it is worth. Boutan (1898: 1887–88) definitely stated that fertilization is not by copulation, but occurs free in the water. This statement is based upon his work on the development of *A. virginea,* but he failed to give his grounds, and the statement is in complete disagreement with that of Willcox. He stated that the eggs are laid singly and immediately washed out into the water, whereas Willcox stated that they are laid in a mucous strand. These contradictory statements may be based on observations made upon different species. The situation is complicated by the possibility that Willcox, as she suggests, may have seen unfertilized eggs laid, and Boutan may have seen eggs which had already been fertilized in the mantle cavity but which did not start development until laid. Thiem (1917: 597) made the statement that he believed internal fertilization to be very unlikely because of the large number of sperm produced, but this is not pertinent evidence. Fischer in 1863 is said (Willcox, 1905: 332) to have made the statement that he had found the ovaries of *Patella* filled with embryos already provided with a shell, which would be strong indication of true internal fertilization. However, he (1863: 321) actually said: " . . . on trouverait l'ovaire des femelles rempli d'oeufs ou peut-être même d'embryons déjà pourvus de coquille." Dall (1879), likewise, believed that internal fertilization takes place, but he is said (Willcox, 1905: 333) to have admitted later to Willcox that his report was based upon Fischer's comments and not upon his own findings. Further, Dall's statement is said (Willcox, 1905: 332) to have been the authority for that made by Tryon in his *Manual.* Thorson (1935) does provide definite evidence that Fischer's ideas were entirely correct, at least as far as the species *rubella* goes. Thus, for one species, concrete evidence has been presented to indicate that internal fertilization must occur.

Because the evidence is about equally divided on this important issue, it is only possible to say that if internal fertilization, or even fertilization only of eggs from females near by (close fertilization), occurs among the species of *Acmaea* in which speciation is presumed to have occurred following ecologic segregation, then speciation here may have progressed by the usually recognized method of gradual selection of adaptive characters. If, however, fertilization should be shown to take place in the water, at any distance from the parents, between eggs and sperm washed about in the currents, then apparently the only possible explanation for the origin of many of the species discussed in this paper would be one something like that suggested by Goldschmidt (1933: 543). A single mutation which would produce changes sufficiently extensive to prevent interbreeding with the parental form would be necessary. If such a mutation occurred in both a male and a female (assuming cross fertilization) in the same locality at the same time (not impossible in the case of animals with such enormous numbers of progeny and such limited dispersal), or took place in the sex chromosome, a new species could thereby be established, with additional changes accumulating in time.

Only in the event of internal or close fertilization would there be any need for ecologic isolation to permit establishment of the new species, but the influence of such isolation would affect the trend of evolution in either case. In the subsequent discussion, arguments in favor of isolation creating the requisite conditions for speciation are understood to apply only if fertilization is internal or close. Since no special conditions would be necessary where speciation has been by the method suggested for distant fertilization, if it ever occurs, no argument is advanced in the individual cases.

Consideration of the manner in which ecologic segregation and selection proceed indicates that this type of speciation may only occur where the ancestral species possesses much adaptability and a wide range of variation, that is, in those species which are eurytopic in nature. Even more obviously, it will usually result in the formation of species of greatly reduced variability. Thus, the tendency in genera in which the type of speciation produced by ecologic isolation has been active must, in general, be from the variable and eurytopic to the nonvariable and stenotopic, whereas in speciation based on geographic isolation there need be no great reduction in either variability or ecologic tolerance, although sometimes there is. There may even be an accumulated increase in variability in races produced as a result of geographic isolation.

ILLUSTRATIONS OF THE SPECIATION PROCESS IN *Acmaea*

As already stated, speciation caused by ecologic and geographic isolation is well illustrated in the genus *Acmaea.* Several examples have been selected and are presented. Speciation based on geographic isolation is best exemplified by polytypic species in which the process of evolution may be going on at the present time. There are at least two polytypic species of *Acmaea* in North America today. In one of them, the geographic races have, in the past, been considered to be valid species, namely *testudinalis* (Müller) and *scutum* Eschscholtz. Actually, these forms can be shown to be two geographic races of the species *testudinalis* (Müller). The race which has been known as the species *testudinalis* occurs along the North Atlantic coasts of Europe, North America, intervening islands, and coasts of the Arctic circle. The race which has been called the species *scutum* Eschscholtz occurs along the Pacific shores of North America from San Pedro, California, to Alaska. North of the Alaska Peninsula, in the Bering Sea, are individuals intergrading in every possible character, individuals which could as well be placed in one as in the other of these two so-called species. Furthermore, examination of the radular ribbon of these two species reveals slight variation. The general pattern is the same, with such differences in proportions and shape as would suggest that differentiation is progressing along different lines in the two groups, but as yet there has been no break or separation between them. Radulae of specimens from the region of intergradation are intermediate in type between the two extremes, a fact which bears out the evidence derived from the shells.

In view of these facts, there is only one possible interpretation of the relationship between these two forms. Instead of two valid species, there are two geographic races of one species. The very extent of this polytypic species, not with regard to number of races but with regard to geographic range, has served to mask its true nature, although more than once it has been stated that the two so-called species undoubtedly were subdivisions of one species (Dall, 1879: 340; Tryon and Pilsbry, 1891: 11).

Acmaea fenestrata (Reeve) is another polytypic species of the Pacific coast of North America. In the northern part of its range, from Alaska to Cayucos, California, the external color pattern is of plain dark slate gray; the aperture is subcircular in shape; the interior is usually suffused throughout with shades of brown. In the southern part of its range, from Point Concepcion, California, southward, the external color pattern is a regular dotting of cream-color pin-point speckles upon a gray-green background; the aperture is definitely oviform; the interior has a small brown apical spot and a narrow brown border, with a blue intermediate area having but little if any brown suffusion. The difference in appearance between these two geographic races is superficially so striking that one would never suppose them to be races of the same species. However, the radular ribbons reveal that they are of fundamentally the same pattern in both instances, with an exaggeration of some features and certain proportions in one but not the other. These are only such differences as might be expected in two diverging lines. Since intergrades between the two races, found between Point Concepcion and Torro Creek, near Cayucos, complete the polytypic-species arrangement, the southern race should be called *fenestrata fenestrata* (Reeve), and the northern race *fenestrata cribraria* (Gould).

These represent the only two polytypic species of the genus *Acmaea* known at the present time in North American waters, although there are several other species which show evidence of having originated by the method of geographic isolation.

Along the Pacific coast of North America are, among many others, four species of *Acmaea* of which the radular ribbons indicate close relationship. These species, *digitalis* Eschscholtz, *scabra* (Gould), *conus* Test, and *limatula* Carpenter, are distinct, since neither shell characters nor radular ribbons ever intergrade. A careful study of the geographic distribution, microhabitat, and adaptive characters of each of these leads to the conclusion that three of them have descended from a polytypic species originally of two races, one of which later split into two additional races (see below) by the process of geographic segregation. The fourth species, *limatula,* appears to be the result of speciation by a process of ecologic isolation and selection (see below). Before discussing the evidence for speciation by ecologic isolation and selection in this group, I think it best to discuss the evidence in favor of the more familiar speciation of *digitalis, scabra,* and *conus* through geographic segregation.

The range of *digitalis* extends from the western extreme of the Aleutian islands, Alaska, to the southern tip of lower California, a distance of more than four thousand miles. The microhabitat, near high-water line, includes the following factors: (1) microscopic algae for food; (2) cracks, depressions, crevices for shelter; and (3) a minimum of submersion. Throughout a great part of the range (Alaska to Puget Sound), this species meets with no competitors of the same genus, but south of Puget Sound it must compete with *scabra,* its nearest relative, which occupies essentially the same microhabitat. *A. scabra* occurs from Puget Sound in the north to the southern tip of Lower California. The numerical occurrence of *digitalis* and *scabra* along their mutual range throws some light upon the situation. *A. digitalis* is very abundant as far south as Point Arguello, California, but becomes progressively less abundant at localities farther south. *A. scabra* is scarce at most localities along the coast north of San Francisco Bay. Equal numbers are present at localities southward from San Francisco as far as Point Concepcion, in which region *scabra* becomes the more abundant species, remaining so as far south as the region of La Jolla. In addition, a third species, *conus* is present at Point Concepcion, California. This species also occupies essentially the same microhabitat as the foregoing two, and competes with them from this point southward. It appears in progressively increasing numbers at San Pedro and Laguna Beach, although it is relatively scarce north of La Jolla. At La Jolla, San Diego, and more southern localities, it is the most abundant species in this microhabitat. Thus, the three species must have arisen by geographic segregation, since they occupy essentially the same microhabitat in three successive areas, but domination in each area is by a different species. In spite of the present extensive overlap of their ranges, the species concerned have different population and range midpoints (Schenk and Keen, 1936: 924–26), and hence probably had at one time three distinct ranges. Perhaps *digitalis* once ranged from Alaska to San Francisco, *scabra* from Monterey to Point Arguello, and *conus* from San Diego southward. Separation could easily have been effected by the existence of such barriers as long stretches of sandy beach, especially if combined with strong outgoing currents, such as undoubtedly existed at the Santa Cruz-Monterey submergence, and probably at the Santa Clara submergence. Either one of these factors alone might have been sufficient to effect local isolation of the forms on either side of the affected area. Under such conditions, geographic races already in existence could have completed their differentiation into species. If the supposed one-time isolation was later eliminated, the subsequent dispersal of the recently differentiated species would account for the present overlapping of their ranges. This is especially probable inasmuch as no one of the species appears to have gained any particular adaptive advantage over the others, except possibly that of greater tolerance to cold temperature (*digitalis*) or warm temperature (*conus*). The present distribution of these species is, undoubtedly, attributable to the fact that *digitalis* is more eurythermal than is either *conus* or *scabra.* This is indicated by a comparison of the distribution of *digitalis,* which is continuous from the Aleutian Islands, Alaska, to the southern tip of lower California, with that of *scabra,* which extends only from Puget Sound to the tip of Lower California, and, in addition, is very spotty throughout the northern end of the range, with numerous populations only where the temperature of the water is higher than in the region in general. Temperature tolerance limitations are likewise indicated for *conus* by its distribution, which extends from Point Concepcion to the tip of Lower California, with the species becoming abundant only where the water temperature is both high and relatively uniform, as at San Diego.

The range of *limatula,* the fourth species of this group, extends from Crescent City, California, to the southern tip of Lower California, and around into the Gulf of California, at least as far as La Paz. It does not, however, occupy the same microhabitat within that range as is occupied by the other three species already discussed. Furthermore, it only becomes abundant at Monterey, California (the occurrence north of that locality is spotty, the species being abundant only where water temperature is high), and remains so as far south as its range extends. Thus, that part of its range in which it is very abundant coincides in part with the region in which *digitalis* and *scabra* compete on equal terms, in part with the region in which *conus* dominates both. It is difficult to conceive how *limatula* could ever have been geographically separated from these related species and yet at present occupy a range which is coincident with part or all of that of each of the other three species.

Consideration of the probable taxonomic relationships of these species to one another makes the problem of their speciation even more difficult to solve upon a geographic basis. *A. digitalis,* the presumable ancestor of the group, lacks pigmentation on the sides of the foot, whereas the feet of *scabra, conus,* and *limatula* are pigmented. In spite of this common character, probably nonselective, these three species do not show evidence of either simultaneous or successive origins. The radular ribbon of *scabra* is most like that of *digitalis,* whereas that of *conus* is midway between the ribbons of *scabra* and *limatula;* the latter is least like that of *digitalis.* The shells of *conus* and *limatula* are most like those of *digitalis* in that all possess a lustrous internal finish, probably a nonadaptive character, whereas *scabra* has a unique opalescent finish of the interior surface. Still more complicating in implication, the exterior of the shell of *limatula* resembles that of *scabra* more than it does any other, since both have ribs elaborated by spinulescent ornamentation, whereas both *digitalis* and *conus* have plain, rounded ribs. These facts appear to indicate that the three species, *scabra, conus,* and *limatula,* must have descended from an offshoot race of *digitalis* which had undergone pigmentation of the side of the foot, and, further, that *limatula* must have split off before *scabra* and *conus* had become distinct, since it has some characters in common with each, and, in addition has a geographic distribution indicating temperature tolerance equivalent to the combined tolerance of the other two species. The implications of these facts make speciation of *limatula* based on geographic isolation seem impossible.

Consideration of the microhabitat of *limatula* throws light upon the problem of speciation, however, and suggests a process of ecologic segregation and selection. *A. limatula,* as mentioned above, does not occupy the same microhabitat as do *digitalis, scabra,* and *conus.* This species occupies what may be described as essentially the intermediate intertidal zone, in contrast to the high intertidal zone in which the other three species thrive. The intermediate intertidal zone provides macroscopic algae as food, in addition to the microscopic film of algae of the high zone. It is the same zone, and an equivalent microhabitat within the zone, as that occupied by *testudinalis scutum. Acmaea limatula* must, therefore, compete throughout much of its range with *t. scutum.* Survival in a microhabitat such as the one occupied by *limatula* depends upon entirely different adaptations than in the microhabitat occupied by the related species, *digitalis, scabra,* and *conus.*

Adaptations for resistance to desiccation are essential to survival in a microhabitat near high-water mark. In species of the genus *Acmaea,* the most usual adaptations toward this end are (1) elevation of the shell, which permits reduction in diameter and hence size of the aperture, and (2) development of a strong homing habit or instinct (Wells, 1917; Hewatt, 1940), which permits growth of the shell to fit the irregularities of the rock, resulting in better retention of moisture. Unfortunately, the value of Wells's findings was greatly limited by the utter impossibility of identifying his species.

In addition to resistance to desiccation, and partly because of the nature of the adaptation to that end, shelter is a great necessity to animals living near high-water line. This need is aggravated by the greatly increased activity of water in this region. The homing instinct helps in this respect, increasing likelihood of the return of the wandering individual to effective shelter. The reduced size of the foot and aperture also assist, since they make available much smaller crevices or depressions.

With regard to the cause of the first of the above adaptations, increase of elevation, Orton (1932) has offered some suggestions. He believed that increased elevation is solely due to constant tension of the foot muscles for maintenance of adhesion and hence is merely a secondary result or corollary of exacting conditions of the environment lasting over a period of time.

Briefly, his theory is to the effect that animals in positions where exposure to desiccation is frequent and of long duration must spend much of their time with foot muscles tightly contracted in order to hold the shell close to the substrate; this action contracts the mantle edge, thus causing deposition of the shell in a small circumference. This limits growth in diameter at the same time that it forces growth in a vertical direction. Animals in locations at which factors enforcing continual tension of the foot muscles are not in evidence may spend much of their time with foot muscles relaxed. In this condition the foot would be spread out, and shell deposition by the relaxed mantle edge would occur in an ever-widening circle, with little or no vertical growth. This theory seems to offer a sound explanation for much of the variation in height-to-width proportions within a species, and for all of such variation exhibited by individuals.

Obviously, it is not the only explanation necessary, since it will not account for the consistent difference in elevation between certain species; for the elevation in such a species as *persona,* which is not only considerably elevated, but also has a very large aperture, nor for the rigid restriction to limited intertidal zone areas of species with certain height-to-width proportions (as elevated *digitalis* to high zone, flat *t. scutum* to a low zone).

These differences indicate that there must be a certain amount of hereditary limitation of the elevation attainable by an individual of any given species, as well as a hereditary tendency toward increased elevation of the shell in certain species, decreased elevation in others. It is only such a hereditary tendency which could limit the vertical distribution of a species in relation to shell elevation. Furthermore, the increased shell elevation and reduced diameter of certain low intertidal zone species cannot be explained as a response to increased tension. *Acmaea insessa* and *instabilis* possess these characters to a marked extent, yet both live in microhabitats in which tight adhesion is unnecessary most of the time, due to the great fluidity of the substrate of floating plants and to the almost total lack of exposure to desiccation in such a location.

In addition, I find it difficult to agree with Orton's statement that "the organism depends upon the surroundings for its characters." As far as an indirect dependence (survival of only the suitably adapted individuals) goes, and as far as development within limits controlled by hereditary tendencies goes, I can agree with him, but after a careful study of the large group concerned here, I cannot admit the unreserved application of the statement.

Individuals of *limatula* with adaptations for resistance to desiccation, with the concomitant increase in height, would have little chance of survival in a habitat where water action is severe. Since desiccation is not a factor in this habitat, individuals with an architecture such as that of *limatula* not only have a chance of surviving, but may be the only successful type. The architectural pattern of *limatula* is characterized by a depressed apex and a large aperture, similar to that of *t. scutum,* the species with which it presumably competes through part of its range. Such a shell offers virtually no resistance to water impact, and at the same time permits a large adhesive surface, releasing the animal from the need for special shelter. Even the lack of any homing behavior, characteristic of these species, is probably an advantage, since the animal feeds when the tide is at flow. At any time when the force of water impact increases unduly, the animal needs merely to clamp down wherever it is, thereby reducing resistance to the water to a negligible minimum at the same time that the full adhesive power of the large foot surface is made effective. Such a behavior trait is, of course, of greater value to a species feeding during submersion than a homing trait by which the animal attempts to reach some more or less distant spot.

Other facts concerning *limatula* and related species make the likelihood of speciation by ecologic segregation and selection even more evident. For instance, a few individuals of the species *scabra* may be found at a much lower vertical level than is the bulk of the population. Such individuals are always those which vary in the direction of a depressed shell architecture, enlarged aperture, and reduced sculpture. It is not hard to imagine that some such extreme variants of a high intertidal zone race, not likely to survive within the normal limits of the parental microhabitat, may have taken hold in a microhabitat in the lower vertical zone, where ecologic conditions were prohibitive to all individuals of the parental species except such variants. Owing to the ecologic exclusion of all but similar variants, the effective though small distance providing isolation, the rigid ecologic elimination of all but similar progeny which must take place, and the very limited motility of individuals, a high degree of selection should ensue, which might fairly rapidly (geologically speaking) result in speciation, providing some of the variations were hereditary. No matter how long interbreeding should remain possible between the old stock and the new, the likelihood would be greatly reduced because of the rigid reciprocal exclusion of each stock from the microhabitat of the other and the small but effective distance separating their respective areas. Such a process of speciation, in which the isolation of the new stock depends upon purely ecologic factors, and/or in which the process of selection of genetic characters has been solely one of elimination by ecologic agencies of unsuitable characters through failure of individuals possessing them to survive, is obviously based on ecologic segregation and selection.

Further examples of speciation by ecologic segregation and selection have been chosen from two other groups of species occurring on the west coast of North America, each group providing several examples. One of these groups appears to center about *Acmaea pelta* as the original eurytopic species. It includes the species *pelta, asmi, instabilis, ochracea,* and *triangularis,* the four last-named being stenotopic divergents from the first, of which the range both north and south extends as far as, or farther than, that of any of the four descendant species. In this connection, the unusual adaptability of *pelta* and its resultant highly eurytopic nature are important, since presumably only from such material could four species of very different ecologic adaptations have arisen.

The geographic range of *pelta* extends from Yokohama, Japan, across the Aleutian Islands, and down the west coast of North America as far as the southern end of Lower California. It is significant that this, the most eurytopic of North American species, has also the most extensive geographic range. *A. asmi,* closely related to *pelta,* is rigidly stenotopic and has a somewhat more restricted geographic range. Its northernmost record is Sitka, Alaska, and I have had specimens from as far south as San Quintin Bay, Lower California. That part of the range in which the species is most abundant is the same territory in which *pelta* is likewise most abundant (Sitka, Alaska, to Point Concepcion, California). Here again is a type of speciation difficult to explain satisfactorily upon a basis of geographic isolation, but which appears logical when considered to be the result of ecologic segregation and selection.

A. asmi is practically limited to existence upon the snail *Tegula funebralis* (Adams) by the peculiarities of its structural adaptations. The snail occurs in the high intertidal zone on rocky flats, in the crevices, and in shallow pools. The shell of *A. asmi* is very small with a small aperture, extreme elevation of the apex, and some lateral compression. Individuals with these traits would be subject to easy detachment by water impact in the *Tegula* habitat, if it were not for the fact that they are almost always upon a snail (F. H. Test, 1945; A. R. Test, 1945*c*) which tends to keep in protected spots, and serves as a shock absorber, partly nullifying the effect of water movement. Since it would only be possible for a very small animal with a small aperture, and hence limited adhesive surface, to maintain position upon the rounded surface of this host snail, animals which grew before sexual maturity to anywhere near the usual size of adult *pelta* would be forced to leave the snail and attempt to find suitable substrate elsewhere before maturity. This would result in automatic elimination of such extreme variants, since suitable substrate for large animals of this type is not available within the limits of motility of these animals. Further, and perhaps of more immediate import, only animals so modified in their feeding habits that they could and would subsist upon microscopic algae could attain maturity in this microhabitat. The reproducing population must have been drawn from those extreme variants of the ancestral species which fulfilled these specifications, all others being automatically eliminated by natural selection, as suggested above. Thus, almost immediate isolation of the occupants of the new microhabitat would result, since the habitat described is not adjacent to that of *pelta*.

The nearly total lack of individual variation in *asmi* indicates that the elimination of unsuitable variants by selection must be rigid. Completion of the speciation process from such extreme and segregated variants, as already suggested, could be very rapid (again, geologically speaking). All specimens of *asmi* are black, with sculpturing consisting of fine riblets and grooves. I have never seen a specimen within the ordinary confines of the species habitat with any external color variation, although specimens with white interiors are occasionally encountered. The latter variation could have no selective value, hence it is not eliminated. The apparently rigid elimination of external color variants, which could only be due to predators hunting by sight, has resulted in protective coloration to the extent that limpets of this species appear to be a part of the host's foot. Possible predators upon limpets include various birds (Henderson, 1927: 178; Bent, 1929: 308, 313, 322; Munro, 1936: 234; Vallentin, 1924: 308–9; Cottam, 1939: 36, 60, 77, 94, 129), rodents, raccoons, and perhaps crabs. These facts make it difficult to explain speciation in this instance by any process other than that of ecologic isolation and selection.

Another species related to *pelta* is *instabilis.* It has a much less extensive geographic range, encompassed by that of *pelta,* and extending only from the northern end of Vancouver Island to Monterey. This species is stenotopic, its microhabitat being limited to the fronds and stipe of the alga *Laminaria Andersonii* Farlow. Individuals of the species are large, as plant-dwelling limpets go, with a rocker-like margin resulting from a marked downward inclination of the sides of the shell as a correlative of extreme lateral compression. This rocker-like margin enables the animal, which moves and adheres lengthwise of the stalk, to fit snugly upon the rounded substrate provided by the subcylindrical stipe of the host plant. The shells of this species are always of a peculiar brown color and horny texture, giving them a striking resemblance to the plant upon which they live. The factors involved in the origin of this species appear to have been somewhat different from those involved in the instance previously described. The substrate plant does not seem to offer any topographical peculiarities which are not likewise offered by *Postelsia,* upon which specimens of *A. pelta* are frequently found. Indeed, if casually examined, the specimens of *pelta* upon *Postelsia* usually structurally resemble the architecture of *A. instabilis* closely enough to be confusing. *Laminaria Andersonii,* however, does not appear to be acceptable food to *A. pelta,* or else the microhabitat represented by it, falling below the low-water line, is too low for *pelta,* or both. Only on the rarest of occasions are specimens of *pelta* taken from this species of *Laminaria.* It is readily conceivable, however, that perhaps at some time when desirable foods were at a mininum, some individuals of *A. pelta* which would or could tolerate *Laminaria Andersonii* as food and were able to stand the greater depth and reduced length of exposure invaded the vacant microhabitat upon this particular plant, and then developed ontogenetically and phylogenetically along the lines necessary for existence upon such a substrate. Evolution of the population of *A. pelta* variants upon *Laminaria Andersonii* along stenotopic lines could have proceeded readily, because of prompt elimination of all variants except the extremes best suited to the microhabitat in question and isolation by the sand around the holdfast from the rocks on which *pelta* is found. The apparent unsuitability of the microhabitat for most members of the parental species would serve as a barrier to interbreeding, assuming close fertilization to take place, since only such variants as those providing the nucleus for the nascent species could survive in the microhabitat. Thus, nearly complete isolation of the nascent species is conceivable.

Equivalent processes can be imagined for the other two species of this group, *ochracea* and *triangularis,* each along its own pecular lines. It seems more profitable to consider the process as it has occurred in another group of species in which the ecologic factors determining speciation have been still different.

The group of related species remaining for consideration centers about the *testudinalis* polytypic species, in particular the geographic race or subspecies *testudinalis scutum.* As already mentioned, the present-day races of the *testudinalis* species are an indubitable product of geographic isolation. Closely related to them are species which have also undoubtedly originated by the method of geographic isolation, such as *schrenkii* Lischke in Japan, *pileopsis* (Quoy and Gaimard) in Australia, and *badia* (Oliver) in New Zealand. In addition, there are several species, *persona, fenestrata, insessa,* and *palaecea,* of which the ranges are entirely or partly coincident with that of *testudinalis scutum* and which appear to have separated by ecologic segregation and selection rather than from a process of geographic segregation.

A. testudinalis scutum occupies a microhabitat essentially in the intermediate intertidal zone, but extending part way into the high and beyond the low intertidal zones. In this microhabitat, the algae *Enteromorpha* and *Ulva* serve most importantly as food. Certain factors are notable for their absence. For instance, danger of desiccation is greatly reduced or absent, permitting development of an enlarged aperture which, in turn, permits depressed architectural structure. Since special provisions for shelter are absent in this microhabitat, the depressed architecture has considerable survival value. Inasmuch as the subspecies *scutum* of the *testudinalis* species is eurytopic and possessed of considerable adaptability, individuals which are structurally extreme radiate beyond the normal confines of the microhabitat, providing material for speciation through ecologic isolation.

Another polytypic species, *fenestrata,* seems closely related to *testudinalis scutum.* This second polytypic species has a geographic range entirely coincident with that of *testudinalis scutum,* extending from Unga Island, Alaska, to San Pedro, California. *A. fenestrata* and *testudinalis scutum,* however, do not occupy the same microhabitat. Both geographic races of *fenestrata* occupy a microhabitat in the high intertidal zone (never extending lower) comprising only microscopic algae as food, and having as active factors frequent danger of desiccation and subjection to severe abrasion because of the large amounts of sand in the water. Such a set of factors requires peculiar adaptations, both of structure and behavior.

Some adaptation to reduce the danger of desiccation becomes essential. This is accomplished in the races of *fenestrata* by a partly reduced aperture and an associated increase in elevation, together with a behavior trait: as the tide recedes, the individuals proceed down the side of the rock until covered by the sand at its base. At night the animals do not move down. Also, occasionally, when the sun is in such a position that the light is evenly distributed on all sides with reference to the animal's location, the limpet fails to move; hence, this must be a weak negative phototropic response. Apparently, the limpet, as the water recedes and the light becomes brighter, moves down the side of the rock, keeping within the area of subdued light. This trait serves, incidentally, to obviate two other dangers. The bareness of the rocks inhabited by this species would make the animals, if exposed by the tide, fairly obvious prey to any predator hunting by sight. Also, maintenance of adhesion during the ebb and flow of the tide, with the accompanying increase in water impact, might be endangered if the animal should remain in an unprotected location on the bare rock. The increased thickness of the shell is probably the most important adaptation for withstanding the abrasive action of the loose sand in the water, aided by the conical shape, and the burying trait at periods when the sand action is most severe.

The species *fenestrata,* then, although it shows no evidence of ever having had a geographic distribution distinct from that of *testudinalis scutum,* seems to have originated within the latter's range. It also exhibits striking ecologic adaptions which may have had their origin in extreme variants of the ancestral race better able to survive in the particular microhabitat occupied by the *fenestrata* group. This microhabitat, untenable to normal individuals of the ancestral race, would provide the requisite isolation for speciation, especially since the increased elevation of the variants would prevent their dispersal back into the ancestral microhabitat. The isolation thus provided should offer the necessary condition for speciation to proceed, and hence here again species formation would be due to ecologic isolation and selection.

Another species, *persona,* which is also related to the race *testudinalis scutum,* offers just as good evidence of speciation by a process based on ecologic isolation. The range of *persona* extends from the Shumagin Islands, Alaska, to Monterey, California, not as far north nor as far south as the more eurytopic *testudinalis scutum.* A study of the ecologic adaptations of the stenotopic descendant, *persona,* again suggests ecologic segregation and selection. The microhabitat of *persona* is very limited in contrast to that of the eurytopic ancestor. The microhabitat, besides being more limited, is very different from that of the ancestral species, occurring in a different intertidal zone. It is limited to the high intertidal zone, comprises microscopic algae as food, and is confined to situations where the light is reduced, such as the undersurfaces of rocky ledges, roofs of caves, and deep crevices.

In locations of this type, certain factors differ decidedly from those affecting the ancestral *testudinalis scutum.* Danger of desiccation is increased because of increased frequency and length of exposure, but the very nature of the locations utilized (see above) limits the extent of this danger. Force of water impact is considerably increased. Danger from predators is greatly reduced by the low visibility. Hence, it is possible for occupants to meet the exigencies of the microhabitat by adaptations other than those encountered thus far, since the differing combinations of ecologic factors change the problems involved in selective elimination. As a result of lack of danger from predators large size is less a liability than in the species previously discussed. Because of some danger of desiccation, increased elevation is desirable. The combination of increased size and increased elevation permits an enlarged aperture, which is not too great a liability in the situation concerned because of protection from sun and wind. Increased elevation, usually an eliminating factor where water impact is great and no special shelter from it is available, is compensated for by other features, namely, large increase in adhesive power due to the large foot surface correlated with the large aperture, and a streamlined architecture. The shells of this species have an outstanding feature, a long, arched, posterior surface which is always oriented to receive the impact of the water. The long arch is structurally sound for the reception of severe stress, and at the same time serves actually to break the force of the impact by its streamline.

In addition, certain behavior traits are essential to the success of the species in this microhabitat. The animals feed only at low tide, and when light at the feeding grounds is very dim. This is feasible since, in this microhabitat, such a set of conditions occurs frequently. With the first persistent splashes of returning tide, or suggestion of increasing light, the animals return to their roosting sites, where they clamp down tightly and remain until conditions are again right for feeding. Since their food (microscopic algae) is abundant and competitors are very few, there is little or no difficulty with regard to obtaining sufficient food in the time available.

Evolution of the architectural structure through ecologic segregation and selection is relatively easy to postulate in this species. The type of sculpture has not deviated far from that of the ancestral race. The general architecture has diverged considerably, but along lines forecast by variants within the ancestral species. The apex is considerably more elevated and somewhat more posterior than that of the ancestral species. The elongated posterior surface is markedly arched, a feature recognizable as an extensive exaggeration of the slight convexity of the posterior face of the ancestral species. The color pattern is more stereotyped both internally and externally. The size is generally larger, and the shells are thicker and heavier. Presumably, any individuals developing along different lines would very quickly be eliminated from this microhabitat. For instance, the flatter individuals characteristic of the ancestral race would die by desiccation during the frequently repeated and relatively long exposures; those with elevation, but no long gently arched surface to ease the water impact would quickly be forced from the rock, or smashed. Individuals with the architectural pattern of *persona* are most unlikely to survive or succeed in other microhabitats. Hence, it is obvious that variants of the ancestral race which were extreme enough to invade such a microhabitat would be truly isolated from the parental species by a large part of the intertidal zone. Speciation could take place by the active selection of the most suitable architectural type. Selection must proceed continually in such a habitat.

DISCUSSION

From these examples, and others not discussed here, it has become apparent that in the genus *Acmaea,* in addition to the process of speciation based on geographic isolation, there has been, and perhaps even more frequently, speciation from eurytopic ancestors by the process of ecologic isolation and selection.

Speciation by ecologic segregation and selection can in this group probably only occur from a eurytopic form, and may presumably take place rapidly as such things go. The materials for it are variants of a given eurytopic species, variants so extreme in their deviation from the normal as to make survival in the usual microhabitat of the parental species difficult or impossible. Such variants may actually thrive, however, if they settle in a suitable microhabitat in which, in their turn, normal individuals of the parental species could not survive. Such a situation may result in the virtual isolation of the variant individuals from normal individuals of the parental species, if there is an untraversable barrier between the two microhabitats, and with consequent inbreeding of the variants if internal or close fertilization occurs. Further, all variants in the progeny which revert toward the ancestral form are bound to be eliminated from the microhabitat by the highly selective action of the ecologic factors involved. The result could be the formation of a new stenotopic species having a tendency, because of ecologic segregation and rigid selection, to diverge from the pattern of the ancestral species by accumulation of the variations successful in the new environment. All those ecologic factors which exercise a selective action upon the individuals living in the microhabitat may contribute to the production of the new species. In the event that external fertilization takes place, and the actual differentiation of a species is due to one mutation without necessarily involving isolation, subsequent ecologic isolation together with selection of the more suitable variants would still play an important part in molding the evolutionary trend of the species.

SUMMARY

It has become apparent that in the genus *Acmaea,* in addition to the process of speciation based on geographic isolation, there has been, perhaps even more frequently, speciation from eurytopic ancestors by the process of ecologic isolation and selection.

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OCCURRENCE OF PARALLEL SERIES OF ASSOCIATED PHYSIOLOGICAL AND MORPHOLOGICAL CHARACTERS IN DIVERSE GROUPS OF MOSQUITOES AND OTHER INSECTS

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INTRODUCTION

SEVERAL characters which are useful for the differentiation of species and subspecies of insects recur in association in widely separated taxonomic groups. Combinations of these characters are present in insects as widely separated as the three major groups of mosquitoes (*Anopheles, Culex,* and *Aedes*), flies of the genus *Drosophila,* and butterflies of the genera *Colias* and *Melitaea.* Knowledge of similar associations between characters in other insect groups is limited only by the paucity of information. Some similar parallel associations are known to be present in other classes of animals.

Most of the physiological characters that are known to be associated are related in some way to the rate of body metabolism. Most of the morphological characters concern differences in size or in proportional development of the body parts. Color characteristics are also involved.

It is the purpose of this paper to illustrate these parallel associations in diverse groups of insects and to discuss the possible mechanisms by which they may have originated.

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KINDS OF CHARACTERS

Combinations of the following characters are associated in a particular order among the various forms of the groups discussed. Owing to differences of opinion in the literature regarding the taxonomic standing of many of these forms, the terms ''species,'' ''subspecies,'' and ''race'' have been avoided in this paper. In their place, the term ''form'' has been used.

A. Development rate. Insect development can be measured in definite units corresponding to the molting periods. In the higher insects there is no growth after the adult stage is reached. It is possible, therefore, to determine the time elapsed for the complete development of the organism or of any part of this life cycle.

B. Activity. In the adult insect it is not possible to measure growth as a characteristic, but it is possible to measure general activity as a quantitative character. This is usually accomplished under controlled laboratory conditions.

C. Mating habits. Some insects mate more readily in small enclosed spaces than do others. Behavior of the former class has been denoted by the term ''stenogamy'' and of the latter class by ''eurygamy.''

D. Ecological distribution. Preference for certain types of environments may be considered a characteristic of an organism in the same way as is any other physiological trait. The environments may be compared as north or south, high or low, hot or cold, dry or humid.

E. Coloration. Usually, only the major color of the insect body is mentioned, that is, the body may be dark or light. The coloration is primarily due to a browning of and the deposition of melanin pigment in the chitin. In the case of pigments of a different chemical origin, as in the Lepidoptera, specific mention is made of the fact.

F. Larval food tolerance. Some mosquito larvae are better able to live in heavy infusions of micro-organisms than are others. This is mentioned as the larval tolerance to strong infusion.

G. Larval tolerance to salt. Some mosquito larvae are better able to exist in water containing salt than are others. This is mentioned as the larval tolerance to salt solution.

H. Adult feeding preference. Some mosquito adults prefer a blood meal from man rather than from other animals, or will attack man more often than do others.

I. Morphological characteristics. The number of hairs emerging from a given locus on the body and the length of the hairs are variable and significant. The relative lengths of parts of the body to one another are also variable and significant. These differences in growth or in body proportions have been mentioned wherever such data are available.

THE ASSOCIATED CHARACTERS IN MOSQUITOES

Culex pipiens

Culex pipiens is one of the common mosquitoes of Europe. The names *molestus* and *pipiens* are used for two forms of this mosquito which are differentiated by certain combinations of physiological and morphological characteristics. These traits are listed in Table I and apply especially to the mosquito strains studied and reviewed by Jobling (1938), Tate and Vincent (1936), and Marshall (1944).

The chitin color of specimens of *Culex molestus* is a "light reddish almost rust color with golden down," whereas that of specimens of *pipiens* is a "dark cigar brown" with the tarsal extremities almost black. *Culex pipiens* is, therefore, darker than is *molestus.* The white knee spots of *pipiens,* noted by Marshall (1944), are a trend toward a paler color. This is, however, a much less significant character than is general body color.

In Europe, the darker form, *pipiens,* exists in the north, the lighter, *molestus,* occurs in the south; there is considerable overlapping in ranges.

C. molestus is a mosquito which, especially in the north, inhabits human dwellings. It is dispersed rapidly by man. In the north *C. pipiens* occupies the natural habitats.

Although *molestus* does not require a blood meal for the development of eggs, it prefers to bite man rather than birds when it does take a blood meal. This fact combined with its domestic breeding habits makes *molestus* a nuisance to mankind. *C. pipiens* prefers birds to man.

C. molestus is able to breed in extremely small enclosed spaces; this permits its engagement in its domestic habits, in which mating often occurs in small containers. The absence of a diapause in this variety shows that it is of a southern or warm climate adaptation. The evidence suggests that it is unable to overwinter under natural conditions in any northern European locality. In central Europe, it overwinters by breeding continuously in relatively warm places such as cellars, cesspits, and animal houses.

Tests on the comparative developing rates of these races made by Jobling (1938) are inconclusive, probably because of the wide temperature fluctuations during breeding of the mosquitoes (as much as 9.5° C. in most of the laboratory experiments). The data do show, however, that specimens of *pipiens* may be able to survive better or for a longer period of time below a mean of 13.5° C. than do those of *molestus*. Larval survival in salt solutions at varying concentrations is greater in specimens of *molestus* than in those of *pipiens;* likewise, survival of the larvae in a more concentrated larval infusion is greater in *molestus* than in *pipiens* (Jobling, 1938).

TABLE I

Physiological and Morphological Differences Between the Two Primary European Forms of *Culex pipiens*

Character	*molestus*	*pipiens*
A. Larval development	Poor survival at low temperatures	Better survival at low temperatures
B_1. Activity at low temperatures (adult)	Poor	Good
B_2. Diapause	Absent	Present
C. Mating habits (comparative size of area necessary for copulation)	Small (stenogamy)	Large (eurygamy)
D. Ecological distribution	Southern (warmer, dryer)	Northern (cooler, wetter)
E. Color (adult)	Light	Dark
a. Dark spots on abdomen	No	Yes
b. Chitin color	Light	Dark
c. White spots on legs	No	Yes
F. Tolerance to strong infusion	Good	Poor
G. Tolerance to salt solution	Good	Poor
H. Food requirements	*a.* Prefers man *b.* Needs no blood for egg development (autogeny)	*a.* Prefers birds *b.* Blood is necessary for egg development (anautogeny)
I. Morphological characteristics:		
a. Number of branches of hairs	Fewer	More
b. Hair length on larvae	Shorter	Longer
c. Comparative length: palpi to proboscis	Palpi shortest	Palpi longest
d. Siphonal index	Smaller	Longer
e. Branches in the siphonal tufts of the larvae (seem to be longer in *pipiens*, however)	More	Less

C. molestus and *pipiens* are not isolated by any known genetic mechanism. When growing in similar places in their natural habitats, cross-breeding and the production of hybrid intermediates occur. Tate and Vincent (1936) summarized cross mating experiments between these two races. Characteristic of these forms was (1) the necessity for a blood meal in order to lay eggs and (2) the ability to mate in a very small space. Neither of these characters is necessarily unit-controlled in heredity; that is, it is possible that a complete series of intergradations occur, the two extreme halves of which have been fitted, more or less, into the classifications as given above. In the F generation, if this is the case, all individuals should be intermediate between the parents. Owing to the nature of the technique it was not possible to discover an intermediate condition.

Tate and Vincent (1936) stated that "in the cross-bred adults stenogamy (mating in small space) appears in most of the progeny, if not all, even in the F_1 generation, whilst autogeny (no blood necessary for the production of eggs) is exhibited by only a small proportion of the progeny of the first three generations." Individuals of *molestus* will mate in any cage large enough to move around in. In the particular experiment mentioned, the egg rafts produced by the F_1 individuals were very small. Specimens of *C. pipiens* produced large rafts and those of *molestus* produced small ones after a blood meal; only *molestus* produces eggs without a blood meal. Perhaps an intermediate condition in this character is suggested in the latter experiment. It is possible, likewise, that the mating behavior would also have been intermediate were a different technique used for testing the character.

The data on nine generations inbred from two intermediate F_1 egg rafts suggest a strong selection in the first two generations for those individuals which mated in a small space and laid eggs without a blood meal. This selection probably continued for the duration of the interbreeding, but showed no effects after the fourth or fifth generations, at which time there was developed a selected strain comparing well with any pure line of *molestus.* This would be expected under the circumstances, in which the selection began with genetically F_1 *pipiens* × *molestus* material and followed the expectations of multiple factor inheritance. Normal laboratory strains remain pure lines of either *molestus* or *pipiens* despite the constant selection in favor of *molestus,* owing to the fact that only a limited genetic variability is available to be selected for in the original strain and selection would have no effect.

Forms having characteristics not identical with those of *molestus* nor *pipiens* recently have been found in Europe (Callot and Van Ty, 1943). These, however, may represent hybridization products of the other two forms.

Anopheles maculipennis

The *Anopheles maculipennis* forms have been of interest since their discovery by Roubaud (1921). There are at least seven forms distinguishable in Europe. These occupy greatly overlapping territories, but are distinct in adult and larval ecologic-niche preferences (Bates and Hackett, 1939). There are distinct morphological differences in the number and branching of the hairs of the larvae (De Buck, Schoute, and Swellengrebel, 1930; Bates, 1939*a*) or in the size of the float in the egg (Missiroli, *et al.,* 1933). The color and pattern of the eggs also serve to distinguish them (reviews, Missiroli, *et al.,* 1933; Missiroli, 1938; Hackett and Missiroli, 1938). It is possible, though not mentioned in the literature, that slight differences in darkness or lightness of chitin in the adults and the larvae exist that are correlated with the egg color characters. Differences exist in types of "mating dance," or rather in premating tactics. These seem to be primarily a matter of the amount of space required for copulation to occur (Bates, 1941). The races have varying larval tolerances for sea water (Bates, 1939*b*), and the adults have varying preferences for taking human or animal blood (Hackett and Missiroli, 1935; and see below). Within any given locality there is a greatly different seasonal distribution of the races (Bates, 1937; and Lewis, 1939). These are dependent upon the seasonal temperature changes.

Data of these sorts have been summarized in Table II, giving for each race a number corresponding to its position with respect to a given character, for example, the lightest race is number 1, the second lightest is 2. Where it has been impossible to derive the relevant data from the literature a question mark has been substituted for the number. Table III is a correlation, showing the number of times that a given race has been indicated by a given number. A clear correlation is shown for most of the characters.

TABLE II

Physiological and Morphological Differences Between the Forms of *Anopheles maculipennis* of Europe

Degree of manifestation of each character except H and I_2 is indicated by the numbers from 1 to 6.

Character	*elutus (sacharovi)*	*labranchiae*	*atroparvus*	*maculipennis*	*messeae*	*subalpinus*	*melanoon*
C. Mating habit (needs small to large cage)	2	2	1	4	5	5	**?**
D. Ecological distribution (from south to north, low to high elevation, hot to cold part of year)	1	2	3	4	5	3	3
E. Egg color (from light to dark)	1	2	3	4	5	**?**	6
G. Tolerance to sea water (good to bad)	1	**?**	2	4	4	3	**?**
H. Adult feeding preference (prefers man = M, prefers animals = N)	M	M	M	N	N	N	**?**
I_1. Egg float size (from small to large)	1	2	3	4	5	**?**	6
I_2. Larval chaetotaxy (actual mean number of branches of antepalmate hairs are given)	31	10	11	13	15	22	24

With *Anopheles maculipennis,* as with *Culex,* these data illustrate that the characters are genetically correlated and seem to be controlled by a multiple factor (blending) inheritance mechanism. The F_1 hybrids are intermediate in mating habits. The larval hair-number characters (chaetotaxy) are also exactly intermediate in the F_1 hybrids (Bates, 1939*a,* 1941; but see below).

As with *Culex,* the lighter forms prefer to bite man. In *Anopheles,* these are *elutus* (*sacharovi*), *labranchiae,* and *atroparvus.* These three mosquitoes are the malaria vectors in the regions in which any of the races occur in the same locality. Bates and Hackett (1939) stated that this man, or nonman, preference terminology is unfortunate, since the former implies a special preference for man over all other animals and the latter a preference for animals over man. Rather than this, *typicus* (*maculipennis*), *messeae,* and *subalpinus* have a narrower range of hosts. All these mosquitoes prefer to bite cattle or rabbits when man is the alternative; the difference between the types seems to be that some are more general in feeding habits than others or that some respond to weaker stimuli than do others. Thus, *atroparvus* and *labranchiae* respond to the weaker stimuli produced by man, whereas the others would not. Nevertheless, Van Thiel (1939) stated that zoophilism *vs.* anthropophilism is a definite smell preference by the mosquitoes.

TABLE III

Correlation Showing the Number of Times That a Given Form Is Represented by a Given Number in Table II

Form	Number of Times						
	1	2	3	4	5	6	Uncertain
elutus	4	1	......	......	......	......	0
labranchiae	......	4	......	......	......	......	1
atroparvus	1	1	3	......	......	......	0
maculipennis	......	......	......	5	......	......	0
subalpinus	......	......	2	......	1	......	2
messeae	......	......	......	1	4	......	0
melanoon	......	......	1	......	......	2	3

It was observed by Van Thiel (1926) that the form inhabiting the brackish-water areas in Holland had shorter wings (*atroparvus*) than did the fresh-water form (*messeae*).

Hackett and Missiroli (1935) reviewed the data on the structure of the spines on the male genitalia of the forms. According to their figures (from material of La Face), the darker forms, *melanoon* and *messeae,* have much thicker and heavier spines than do the lighter forms, *atroparvus* and *labranchiae,* whereas the intermediate type, *maculipennis,* itself is intermediate. Thus, details in male genitalia may be ecogenotypically as variable as any other end product of a complex development.

These authors provide illustrations of variation in structure of a hair of the second abdominal segment of the larvae. The hairs on the darker forms (*melanoon* and *messeae*) are greatly expanded and developed, in the lighter forms (*labranchiae* and *atroparvus*) they are short and stubby, whereas in the intermediate form (*maculipennis*) they are intermediate. These differences agree with those of the antepalmate hairs described below and the male genitalia spines described above.

Data on the branching of the antepalmate hairs of the various *maculipennis* forms have been summarized by Bates (1939*a*). These data do not agree completely with those obtained on the other characters, unless the forms are divided into subsections, as has been suggested by Hackett and Bates (1939), Bates (1940), and others, who place *sacharovi* as a specific unit separate from the *maculipennis* complex. These sections are: (1) *sacharovi* (*elutus*), (2) *subalpinus* and *melanoon,* (3) *labranchiae, atroparvus, maculipennis,* and *messeae.*

Within each of these divisions, the number of branches on the antepalmate hairs follows the same pattern of variation. Likewise, most exceptions to the rule as noted for the other characters are eliminated.

Hybridization of some *A. maculipennis* forms led to the determination that the F_1 hybrids are intermediate between the parents in the branching of the antepalmate hairs (Bates, 1939*c*). Hybrids tested were as follows (the numbers represent the hair number): *atroparvus* (11) × *subalpinus* (22) giving F_1 (16); and *atroparvus* (11) × *sacharovi* (31) giving F_1 (17).

In geographical and ecological distribution, each form of the group is distinct, but overlaps some one or more of the others. Despite this overlap, there seems to be little natural hybridization among them. The data of De Buck, Schoute, and Swellengrebel (1934) and Bates (1939*c*) suggest that intercrossing would be rather ineffective in transferring genes from one population to the other. Sterility of one sort or another seems to result from intercrossing; yet in some instances hybrid individuals are fertile and give rise to fertile progeny in backcrosses.

In distribution the darker forms are more northern and the lighter are more southern. Where the two occur in the same general locality, for instance in Albania, the darker are commoner at the higher and cooler elevations than are the lighter. There is a definite segregation of the races latitudinally and climatically, even though a tremendous overlap occurs (Lewis, 1939; Bates and Hackett, 1939). The distribution of *melanoon* and *subalpinus* seems less definitely known than that of the others and, on this account, their distributions are more difficult to correlate.

In the several parts of Albania, there is a great change in the frequencies of the forms at various times of the year (Lewis, 1939). At the sea-level localities, *subalpinus* and *maculipennis* (both intermediate in color) are most abundant in the spring; there is an increase of each in population as the season progresses. There is, however, an even greater increase of *sacharovi* (lightest colored). At an intermediate elevation in the mountains *maculipennis* is most abundant, but in the warmer part of the year the population increase of *subalpinus* and *sacharovi* is comparatively greater. At the locality of highest elevation, *maculipennis* is most common early in the season, but is largely superseded during the warmer season later by *subalpinus.*

Ecological segregation also exists with respect to the type of breeding place selected by a form. For example, in northern Europe *atroparvus* occurs along the coasts, usually in brackish water; in the south, however, it breeds in fresh water and at inland localities. *A. labranchiae* is confined to the coastal areas of the Mediterranean west of the Adriatic; this is the part having the warmer winter temperatures. In the southern parts of its range it breeds in all habitats; in the north, breeding is only in brackish water. *A. messeae* is a continental type, breeding only in fresh water and preferring situations where the winters are cold. *A. sacharovi* is the Near East form occurring in the Balkans and along the coast of Italy, in Albania only in the coastal marshes. In the hottest part of the summer, it ranges inland. *A. maculipennis* itself is the most widely distributed of all, but it is ecologically intermediate in its preferences. It gives way to *atroparvus, sacharovi,* and *labranchiae* in the south and to *messeae* in the north. In Albania, during the warm season, it is replaced by *sacharovi* at the lower elevations and by *subalpinus* at the higher elevations.

The evidence suggests that the races which seem to prefer brackish or salty water in any region may not actually prefer it, but are merely able to tolerate it. They may live in brackish water within an area because it may be the only place available in the vicinity not already occupied by another form better suited for competition there. For example, in the northern parts of the distributions of each of the southern forms, *atroparvus, labranchiae,* and *sacharovi,* the larvae breed in salt water. In the southern parts of their ranges in the absence of the more northern races, they also occupy fresh water. The range of each of these forms is different. Thus, three factors may regulate the coastal, brackish-water distributions of these southern mosquitoes in the north: (1) the colder winter temperatures inland, (2) the competition inland with the northern forms, and (3) the better tolerance to salt water of the lighter or more southern forms.

In sexual behavior, the forms align themselves in order corresponding to the color types. These are shown in Table II (from De Buck, Schoute, and Swellengrebel, 1930; Corradetti, 1937; Bates and Hackett, 1939; Bates, 1941). The observation in *Haemagogus* (Hovanitz, 1946*a*) that this behavior is of degree only and therefore is likely regulated by a complex hereditary mechanism is illustrated by the results of a cross between a form that will mate in a cage of any dimension (*atroparvus*) and one that will mate only in a very large cage (*typicus* or *maculipennis*). The hybrid individuals swarm (the preliminary to mating) in the same size cage as the form *labranchiae*. *A. labranchiae* is intermediate between *atroparvus* and *maculipennis.*

Haemagogus

Haemagogus mosquitoes are closely related to the *Aedes* group. Eight forms are present in the northwestern part of South America. Most of these have different geographical distributions. In some places as many as three forms occur in the same forest patch, others seem to be replaced geographically by another form (Kumm, *et al.*, 1946).

Judging from geography alone, one may divide these *Haemagogus* forms into the following groups: (1) *falco,* (2) *equinus, andinus,* (3) *splendens, lucifer, boshelli,* and possibly *chalcospilans,* (4) *anastasionis.* By qualitative morphology, these same groups are apparent (Table IV).

H. anastasionis, chalcospilans, equinus, and *lucifer* occur in various parts of Central America, but not south of Colombia in the same form. *H. andinus* occurs at higher elevations in a cooler climate than do the other species. *H. lucifer, splendens,* and *boshelli* occupy contiguous geographical areas without overlapping. *H. splendens* occupies all the hot area in northeastern Colombia and adjacent Venezuela, which is characterized by a very long dry season; *lucifer* occupies the more central parts of Colombia; *boshelli* occupies the rainy coastal area of the Chocó, where no dry season seems to exist.

TABLE IV

Comparison of Some Qualitative Characters of Colombian *Haemagogus* Mosquitoes

Character	*splendens, lucifer, boshelli, chalcospilans*	*equinus*	*andinus*	*anastasionis*	*falco*
Antennae of male heavily or lightly plumose	Light	Heavy	Heavy	Heavy	Heavy
Palpi length	Short	Long	Short	Short	Short
Tarsal claws of female toothed	No	Yes	Yes	Yes	Yes
Postnotal setae	Present	Absent	Absent	Absent	Absent
Second marginal cell on wing longer or shorter than petiole	Longer	Shorter	Shorter	Shorter	Shorter
Comb scales on larvae	Patched	In line	In line	In line	In line
White spots on femora	Absent	Present	Present	Absent	Absent
White scales on thoracic lobes	Absent	Present	Absent	Present	Present
Sternopleural setae of larvae	Absent	Present	Present	Absent	Absent

H. falco reaches its most northern limit in Panamá. Geographical differences are apparent within this group: the populations of eastern Colombia are different from those of the western area (Table V). *H. anastasionis* has a sporadic distribution in Colombia. Quantitative morphological and physiological characters separating these *Haemagogus* are given in Table V.

For the physiological characters, numbers are given to each species, as in *Anopheles,* to represent the relative position with respect to the other races in the quantitative characterization of the racial features. In the group *splendens-lucifer-boshelli, splendens* is at one extreme, *boshelli* at the other, and *lucifer* is in the intermediate position. Each character corresponds to a similar one in the *Anopheles* group. Of the other *Haemagogus, H. equinus* is the second lightest in color and generally has second place in the table. *H. andinus* is the darkest, with the possible exception of *boshelli,* and is generally in the last place. In the *equinus-andinus* group, *equinus* takes precedence over *andinus*. *Haemagogus anastasionis* is an intermediate entity, though it is unique. *H. falco* also stands apparently unique, but it is darker than is *anastasionis*.

As in *Anopheles* there seems to be a constant seasonal change in the frequencies of the forms in the same locality as well as regularities in the spacial distribution. For example, at a place called Volcanes, where *lucifer*, *equinus*, and *falco* occur together in the same forest, Boshell recorded for May and June, 1943, all the males as *lucifer* (Kumm, *et al.*, 1946). In October, 1943, during a dry period, 54.8 per cent of the males were *equinus* and 45.2 per cent were *lucifer*. No males of *falco* had to that date been obtained in the wild. In the same May and June above, 88.3 per cent of the females caught were *falco;* in October and November, most were *equinus*.

TABLE V

COMPARATIVE RATINGS OF PHYSIOLOGICAL AND MORPHOLOGICAL DIFFERENCES BETWEEN THE RACES AND SPECIES OF COLOMBIAN *Haemagogus* MOSQUITOES

	splendens	*lucifer*	*boshelli*	*equinus*	*andinus*	*anastasionis*	*falco*
A. Development rate (fastest to slowest)	2	4	5	1	?	?	3
B. Activity of adults (25–28° C.)	1	3	3	2	4	?	5
C. Ease of mating in cages (small to large)	1	3	3	2	4	?	5
D. Ecological distribution (hot-arid to cool-humid)	1	4	6	2	7	3	5
E. Adult coloration (light to dark)	1	5	6	2	7	3	4
F. Larval ability to withstand strong infusion (strong to weak)	1	2	3	1	3	?	2
I. Morphological characteristics: (mean numbers)							
Upper posterior pair of head hairs	1	1	1	1	2	1	1
Lower anterior pair of dorsal head hairs	2	2	2	1	1	1	1
Hairs in ante-antennal hair tuft	3	5	5	3	8	3	3
Scales in comb	18	24	34	8	10	8	7
Scales in pecten	16	13	14	12	17	15	10*
Hairs in lateral tuft on anal segment	2	3	2	2	5	5	2*
Ratio: siphon length to width	1.5	1.5	2	2	3	2	1.5
Scales in pecten tuft	3	3	4	2	3	3	2*
Setae tufts on side piece, male genitalia	0	1	3	?	?	?	?
Setae on venter of male adult	0	?	?	Few	Many	?	?

* These scales and hairs are longer in *falco* than in *equinus;* in the case of *falco*, there are four hairs instead of two in material from Leticia in far southeastern Colombia; likewise in the case of the ante-antennal tuft there are three hairs in the material from east of the Cordillera Oriental and two in that from west of there.

In another locality where the rainy season catch is *andinus*, during the dry season only specimens of *anastasionis* were obtained. The trend seems to be toward the darker form during the rainy, cloudy, or cooler part of the year and toward the lighter forms during the dry, sunny, and warm part of the year. This is similar to the trend noted for *Anopheles maculipennis.*

Anopheles albitarsus

A species or variety of *Anopheles albitarsus,* characterized by its domestic habits and by its fondness for taking human blood, exists throughout the central lowlands of Brazil in the vicinity of Rio de Janeiro. It is a notorious malaria vector (A. L. Ayroza-Galvão, personal communication). This race has been given the name *Anopheles albitarsus domesticus.* At the higher elevations at São Paulo, Brazil, there exists another variety (*A. albitarsus albitarsus*) which is not an effective vector of malaria; it lacks domestic habits and feeds rarely on human blood. This race also lives throughout the temperate parts of Argentina to the south. It apparently prefers cool living conditions in contrast to *domesticus,* which occupies the tropical lowlands to the north. In body characters the two races are distinguished by the extent of melanization of the tarsus; in specimens of *domesticus* the second hind tarsus is only about 50 per cent melanic, whereas in those of *albitarsus* (typical form) the corresponding structure is about 70–95 per cent melanic. Therefore, the character correlations compare with those in other mosquitoes.

THE ASSOCIATIONS BETWEEN CHARACTERS IN OTHER INSECTS

Kühn and von Engelhardt (1937) have shown that a genetic mutant of a moth in which the wings are suffused with melanin pigment has greater viability under conditions of lower temperature and increased humidity than has the same moth without the mutant effects. It also has a slower development rate. This is a pleiotropic effect of a single gene.

For many insects the data which point toward a correlation between body color and ecological living preference, especially in the Lepidoptera, have been received earlier (Hovanitz, 1941). The correlations correspond to those in the mosquitoes.

The genus *Drosophila* has similar correlations. Table VI shows the correlation between the development rate of twenty-two species of Japanese *Drosophila* and the amount of darkness which these species exhibit in their body color. Darkness is associated with slow development rate and lightness with rapid development rate. Correlations with the nature of the environment as judged by northern versus southern distributions also have some significance. The average length of development for all species listed by Kikkawa and Peng (1938) as Arctic or temperate in geographical distribution is 14.6 days (thirteen species); for those with a tropical distribution, the average is 10.5 days (seven species). The growth rates were tested in the laboratory at a comparable temperature of 26° C.

The comparative rates of development between some closely related *Drosophila* species or races which differ in chitin color may be illustrated by *D. pseudoobscura* (race A), *D. pseudoobscura* (race B or *persimilis*), and *D. miranda.* These species or races occupy contiguous or overlapping geographical regions from south to north in the order given. *D. miranda* is the darkest in color, whereas race A of *pseudoobscura* is the lightest; races A and B are not obviously different in this respect. Under the same conditions, *D. miranda* develops the slowest of the three. *D. pseudoobscura* race B (*persimilis*) is second, and race A develops the fastest (Poulson, 1934; Dobzhansky and Epling, 1944).

These differential developing rates, colors, and ecological preferences are associated with some other physiological characteristics. At 25° C., race A deposits more eggs than race B; at 19° C., both races are about equal in fecundity; and at 14° C., race B is superior to race A (Dobzhansky, 1935). The life expectancy of race A in the absence of food is greater than that of race B (Lilleland, 1938). This may be an indication of a greater resistance to desiccation in race A than in race B. Apparently, there is a slightly different rate of oxygen consumption in the two forms (Dobzhansky and Poulson, 1935). The frequency of wing beats per unit time in flight has been found to be greater in race A than in race B (Reed, Williams, and Chadwick, 1942). This may be an index of activity of the adults. Mather and Dobzhansky (1939) have found that the number of sex comb teeth is slightly greater in A than in B and that the wings of B are slightly larger than those of A. The wings of specimens of *miranda* and the body size in general are larger than in either race of *pseudoobscura;* also the teeth in the sex combs are more numerous than in *pseudoobscura.* These characteristics, except for sex comb number in races A and B, are parallel to corresponding characters in the mosquito races. These data are summarized in Table VII.

TABLE VI

CORRELATION BETWEEN DEVELOPMENT RATE AND COLORATION OF ADULTS IN JAPANESE *Drosophila*

Development data from Kikkawa and Peng (1938); classification into arbitrary color classes by A. H. Sturtevant (personal communication). Arbitrary scale of pigmentation: lightest = 1, darkest = 5.

Development Days at 26° C.	1	2	3	4	5
10	2	3	0	0	0
12	2	5	0	1	0
14	1	1	1	1	0
15	0	0	0	1	0
16	0	0	0	1	0
18	0	0	0	1	0
20	0	0	0	1	0
25	0	0	0	0	1

In the races and color forms of *Colias* butterflies in North America, correlations of the same type between genetic characters of growth and form, color, and geographical distribution are present. The correlations between color and form hold whether races (or close species) are considered (Hovanitz, 1944*ab*) or whether a single genetic mutant within a race is considered (Hovanitz, 1944*c*, 1945*a*). Color, ecological distribution, laboratory environmental preferences, and development rate so far as is known are similarly associated. With *Colias,* it was possible to illustrate by experimental manipulation of the environmental conditions how the resulting change in metabolic rate affects directly the morphological and color characteristics of the organism (Hovanitz, 1946*b*). As in the mosquitoes, the race with the higher metabolic rate and southern-type characteristics has been better enabled to become a pest to mankind (Hovanitz, 1944*d*).

TABLE VII

Comparative Physiological and Morphological Data on the *Drosophila pseudoobscura* Group

	pseudoobscura A	*pseudoobscura B* (*persimilis*)	*miranda*
A. Development rate (fastest to slowest)	1	2	3
B_1. General activity	High	High	Low
B_2. Frequency of wing beats (high to low)	1	2	?
B_3. O_2 consumption (probable) (higher to lower)	1	2	?
D_1. Ecological distribution (warm-dry to cool-wet)	1	2	3
D_2. Fecundity best at temperatures	High	Low	?
D_3. Life expectancy without food (good to bad)	1	2	?
E. Color (light to dark)	1	1	2
I_1. Number of teeth in sex combs (low to high)	2	1	3
I_2. Wing size (small to large) ♂	2	1	3
I_3. Tibia length	2	1	?

The races of *Melitaea chalcedona* exhibit a similar array of characteristics (Hovanitz, 1942, and unpublished observations). The darkest races inhabit ecological areas having cooler and wetter environmental conditions; the lightest races inhabit ecological areas having hotter and dryer environmental conditions. Laboratory observations indicate that the light races will mate more easily in small cages than will the dark. Likewise, the developmental rate of the lighter is more rapid than that of the darker.

DISCUSSION

The preceding examples illustrate the occurrence of a parallel series of associated physiological and morphological characters in several different species groups of mosquitoes and other insects. The associated characters are various qualities of (A) development rate, (B) activity of adults, (C) mating habits, (D) ecological distributions, (E) coloration, (F) larval food, (G) salt tolerances, (H) adult feeding preference, and (I) morphological characteristics.

In each instance where information is available (Table VIII), that is, for *Culex pipiens* forms, *Haemagogus* forms, *Drosophila* species, *Drosophila pseudoobscura* forms, *Colias* forms, and other Lepidoptera, there is a range in variation of the species from those with a slow to those with a fast rate of development. These differences in the rates of development are correlated with the variation of certain other characteristics. For example (Table VIII), some forms of *Culex pipiens, Haemagogus, Drosophila pseudoobscura, Colias,* and other Lepidoptera have, in association with the rapid development rate, a greater adult activity. In *Culex* and *Colias* the existence of a diapause is considered an indication of a lowered activity. The members of the *Drosophila pseudoobscura* group exhibit greater activity by a higher oxygen consumption and by a higher frequency of wing beats. Observable differences in activity are also evident to the laboratory observer. The greater fecundity of the one race (A) at a higher temperature than of another form (B) may be an indication of activity differences at the temperatures compared. Motion or flight activity in captivity in other groups has been observed directly.

TABLE VIII

THE INFORMATION AVAILABLE IN THE SPECIES GROUPS AND THE DEGREE OF THEIR FIT IN THE CORRELATIONS

Key: +, information available and a fit with the variation expected.
Blank, no information available.
±, information available, data in agreement, but uncertain, or not directly applicable.
+*, a partial disagreement with the direction of variation expected in the group, but the general trend being in agreement.
+°, an apparent disagreement, due to different pigment involved.
0, information available, but no positive or negative fit.

	Culex pipiens	*Anopheles maculipennis*	*Anopheles albitarsus*	*Haemagogus*	*Drosophila* species	*Drosophila pseudoobscura*	*Colias*	Other Lepidoptera
A	±			+	+	+	±	+
B	±			+		+	+	+
C	+	+*		+			+	+
D	+	+	+	+	+*	+	+	+
E	+*	+	+	+	+	+	+°	+
F	+			±				
G	+	+						
H	+	+	+					
I	+*	+		+*		+*	0	0

The members of many groups of insects differ in their ability to mate in a small enclosed space. Some forms require very little space for flight. Others require a large area before the sexes will be attracted or will copulate. In each group a series of forms requiring from little space to large space may be arranged. In each of the groups, *Culex pipiens, Anopheles maculipennis, Haemagogus, Colias,* and *Melitaea,* such a series may be arranged. Each of these falls into concomitant association with the other physiological characters, namely, rate of development and activity. There is, however, a discordant species in the *Anopheles maculipennis* group concerning mating habits.

The ecological distributions of the members of these species-groups differ in that some forms exist in a cold or damp climate and others in a hot and dry one. Each group (Table VIII) forms parallel series with the other groups in regard to this character. Species which prefer cold and damp climates are sluggish (less active), have a slower development rate, and need a large space for mating. Owing to the fact that the relationships hold well only for closely related members of a group, the general species of the genus *Drosophila* possess this relationship in a statistical sense only. Others, such as the *Anopheles maculipennis* group and *Haemagogus* show it best when subspecies rather than species are compared. The greater the phylogenetic diversity the less the amount of regularity which can be expected in direct comparisons.

In the darkness of melanin pigmentation or of chitin coloring, the members of the groups fall into series which vary concomitantly with other characters. Darkness of pigmentation is associated with a cold and damp climate, a sluggish behavior, a slow development rate, and the necessity for a large space for mating. In *Colias,* a pigment of a different chemical nature from melanin gives a reversal to the usual associations for color. With respect to melanin in this genus there is no reversal. In *Culex pipiens,* there is a partial lightening of color where a darkening might be expected, but the general body is dark, as expected.

The relationships of the tolerance to various concentrations of larval food and salt solution have been determined in only three groups (only two thoroughly. In *Culex pipiens,* various concentrations of salt solutions and of density of micro-organism infusions have been tested. In *Anopheles maculipennis* only salt solutions and in *Haemagogus* only infusions have been tested. In all three cases the forms in a group which can withstand the most concentrated solutions and infusions are those which have the fastest development rates, have the greater activity, can mate in a small space, prefer hot or dry environments, and are pale.

The characteristic concerned with the adult feeding habits can be applied to mosquitoes only. For a blood meal these have a choice between man and animals. In each of the three groups on which information is available, namely, *Culex pipiens, Anopheles maculipennis,* and *albitarsus,* the species which bite man are those which also have the characteristics enumerated in the preceding paragraph (see also Table VIII).

Associated with these physiological characteristics are certain morphological ones which have been discovered to aid in the differentiation of closely related species. They are generally body-size characters or differences in specific proportions and amount of development of body protuberances or appendages. In *Culex pipiens,* these characters are: number of branches to the hairs, hair length on the larvae, palpi length in relation to the proboscis, relative length of the siphon, and the number of branches in the siphonal tufts of the larvae. In relation to each of these characteristics one form might be described as having fewer or being shorter or smaller, and the other as having more or being longer or larger. Thus, all characteristics vary in parallel, except the number of branches in the siphonal tufts, which is greater when the other characters are on the decrease. In compensation for this exception, the length of these tufts is shorter when the number of branches increases.

The morphological characters which are considered in *Anopheles maculipennis* are the number of branches of antepalmate hairs in the larvae, the relative length of the wings to the body, the robustness of some spines on the male genitalia, and the expansion of hairs on the second segment of the larvae. In all instances, when the group is divided into three natural parts, there is a correlation between increased number of hairs, length of parts, robustness of parts, and expansion of hairs.

The morphological characters of the *Haemagogus* mosquitoes fall into the same general classes. Four different sets of hairs have been counted. The number of scales in the comb and pecten and in the pecten tuft of the male genitalia and the ratio of the siphon length to its width have been analyzed. With few exceptions, when the number of hairs or setae increases at the different loci (seven loci), the number of scales increases at the other loci (two loci) and the appendage length increases (siphon length). In the three cases in which exceptions have been encountered, the exceptions have been compensated for by increases in other ways, that is, if the numbers of hairs or scales have decreased where an increase is expected, an increase in length may compensate (Table V).

Only three morphological characters are analyzed in *Drosophila pseudoobscura,* namely, number of sex-comb teeth and the size of the wing and tibia. As these measurements were not comparable with some part of the body, they are difficult to interpret. The variation of all characters is parallel in these three forms (Table VII).

In *Culex pipiens, Anopheles maculipennis,* or *Haemagogus* a corresponding type of morphological variation has been shown to be associated with corresponding types of physiological variations. The increase in the numbers of hairs or scales or in length of body appendages or hairs is correlated with a slower development rate, a sluggishness of the adults, a requirement of greater space for the copulation flight, a preference for cooler and wetter environments, a darker melanin coloration, a poor larval food and salt tolerance, and an adult preference for animal blood. Races A and B of *Drosophila pseudoobscura* exhibit an indirect relationship which is difficult to explain, since *D. miranda* shows a direct one. But this may be explained below.

Interpretation

The interpretation of these phenomena must be based upon biological or natural processes at two levels. The first level is marked by the association of different characters in a species group of animals and their concomitant variation within that group. At the second level is the repeated variation or parallel association of these characters in discrete species groups. With an explanation secured for the first level, the second will readily be understood.

An investigation into the developmental mechanics of the characters discussed above may throw some light on the meaning of their association. One is handicapped here, however, since the information in these fields is very meager. Most of the fundamental principles governing the developmental physiology of the characters under discussion are still unknown. Without a knowledge of the interrelationships in development of these various characteristics, most hypotheses will fall far short of their goal.

The associations of the characters described above and their concomitant variation within a species group might be due to one or other or both of two genetic mechanisms. First, all the characters may be controlled in development by the same gene or by a closely linked group of genes. This would be an example of the pleiotropic or manifold effects of genes for which increasing evidence is becoming available. It would seem illogical to attribute all the associations between the characters of these animals, however, to such a mechanism. The second possible interpretation is independent of the first. Each character might be independently regulated in development and happen to be associated because of selection by the same factors of the environment. The evidence indicates that each of these possible mechanisms probably plays some part in the development of the associations described.

Some of the best evidence supporting the pleiotropic nature of many of the associations has been summarized by Neel (1940) from other authors, all pertinent parts being reproduced in Table IX. This table shows the correlation between character expression and increased temperature and increased body size in *Drosophila melanogaster* mutants and wild type. The primary effect of temperature on the body processes in insects is to increase or decrease the body activity. An indirect relation may then be made between

TABLE IX

A Comparison of the Correlation Between Character Expression and Higher Temperature and Greater Body Size (*D. melanogaster*)

Character	Higher Temperature	Greater Size
Wild-type wing length	Decreased	Increased
Fore-femur length	Decreased	Increased
Number of teeth in wild-type sex comb	Increased	Increased
Vestigial wings	Decreased	Increased
Scute bristles	Some increase Some decrease	Same

increased activity and character expression. The wild-type wing length was decreased by higher temperature, but also increased with increase in body size. The fore-femur length was decreased by higher temperature and increased by larger body size. The number of teeth in the wild-type sex comb was increased with a higher temperature and also increased with larger body size. The size of vestigial wings was decreased by increased temperature and increased with increased body size. The number of scute bristles was increased in the case of some strains and decreased in others.

Comparison of these results with the associations of characters in wild forms described in this paper shows significant correlations. *Drosophila pseudoobscura* (Table VII) shows an increase in number of teeth in the sex combs in relation to the size of the form; that is, race B is the smallest and has the smallest number of teeth, race A is intermediate, and *D. miranda,* the largest in size, has the greatest number of teeth. Tibia length, wing size, and number of teeth in the sex combs also are directly correlated. Since the correlation of these characters is probably independent of some of the other characters, the misfit of these data as indicated in Table VIII is not illogical. As the sex comb teeth number is also correlated with increased temperature (increased activity), race A is not out of place in having a larger number of teeth than race B; *miranda,* however, is. One cannot expect the characters to fit two opposed factors.

The relation of activity and temperature to bristle number increase or decrease is variable. Nevertheless, in most stocks there were more bristles induced at lower temperatures and fewer at the higher temperatures (Child, 1936). There is a direct correlation between the size of the body and the number of chaetae at various places on the body (Neel, 1940 and 1943), the larger the size, the more bristles at any locus. Body size is indirectly correlated with temperature in *Drosophila;* large flies are produced at lower temperatures and smaller ones at higher (Stanley, 1935; Combs, 1937). Thus, at the lower temperatures more bristles should be produced. Ives (1939) found more bristles in a scute mutant at high temperatures and less in a wild type. But it is a wild type with which we are concerned.

A comparison of these correlated developmental phenomena shows that the forms which exhibit a more rapid development rate and greater activity are also those forms which have fewer parts in or a lesser development of the hairs, appendages, etc. (*Culex pipiens, Anopheles maculipennis, Haemagogus, Drosophila pseudoobscura*). In most of the forms with an increase in bristle, hair, or chaetae number or length, there is a parallel variation of these units in all parts of the body. This is present despite the fact that different loci are under different genetic regulation as judged by their independent variations (Ives, 1939; Helfer, 1939; and Neel, 1941). There is however, in polychaetoid *D. melanogaster,* a relation between the number of bristles present at a body locus and their total length (Neel, 1940).

There are numerous instances, reported even in old literature, in which increased temperature and activity result in a paling of the colors of the body. This relationship holds generally for chitin tints and melanin deposition. A specific instance has been studied in *Colias* (Hovanitz, 1946*b*), in which another pigment (pterine) behaves in an inverse fashion from that of melanin even though at the same time melanin varies according to rule. It would be an oversimplification and would not basically solve the problem to suggest that metabolism and color of the pigment may be directly related to general body activity. General activity may be increased either by a temperature increase or by a genetic mechanism. The result in either case would be physiologically comparable, leading to an organism having all properties adjusted to a more rapid rate of development.

The correlation of these characters with the natural environmental conditions follows the basic developmental pattern. Those species existing at the higher temperatures have the characteristics expected of forms with greater metabolic activity, and those which live at the lower temperatures have the characteristics expected of forms with lower metabolic activity. There is nothing strange about these relationships. Nevertheless, but little seems to be known as yet of the fundamental principles of development by which these relationships are produced. Until it can be shown definitely that there is a selection for the forms which possess a higher metabolic level in the ecological areas where such a level is advantageous, such a deduction must remain unproved. The Lamarkian hypotheses need not be invoked; neither, on the other extreme, need the hypotheses of some yet unknown character be postulated.

Associations of characters of these sorts are not restricted to insects. Similar relationships have been pointed out in various laws of geographical variation in vertebrates. Apparently, pleiotropic effects are not uncommon for certain of the genes that regulate the characters of color, activity, and size of body parts of animals. The pleiotropic effects are not yet demonstrated beyond question in many cases, but they may be looked for now that their existence is expected. In mice, MacArthur (1944), and in rats, Keeler (1942), Keeler and King (1942), and Shopbach, Keeler, and Greenberg (1943) have shown that some genes may have pleiotropic effects. Correlations of these genetic characters with the environments of the animals have not been made. Field work has shown color and size characters in correlation with the environment. Laboratory work indicates a developmental relation between activity, color, and body proportions. A more direct correlation between the three latter characters and the environment is to be looked for.

From the standpoint of medical entomology it is interesting to note that it is those species of mosquitoes which have characteristics correlated with greater activity which are the pests of man and the carriers of disease. For example, the form *molestus* in the *Culex pipiens* group is the species which inhabits houses and prefers to bite man rather than animals. Several of its characteristics make it well able to do this. A more rapid development rate is advantageous in a temporary pool where the larvae are breeding. The ability to mate in small containers is advantageous, when only such small containers are available in houses and barns. The greater activity of the adults and their preference for a blood meal from man are advantageous in a rapidly changing environment of this sort.

Anopheles maculipennis forms of this type are also those which bite man and which transmit malaria in the regions in which they exist. These are *elutus, labranchiae* and *atroparvus.* The *Anopheles albitarsus* form having corresponding characteristics is also a pest to man and the carrier of malaria in its region.

The evidence presented suggests that the parallel association of distinct characters in these discrete groups of organisms may be due to one or both of two genetic mechanisms. First, though the characters may be distinct in form they may be connected in development by a pleiotropic or manifold effect of the same developmental processes so that when one character varies the other also does. Second, several characters may be selected by the same environmental factors, leading to a corresponding association in the same organism.

SUMMARY

1. The association of several characters has been shown to occur in discrete species groups of mosquitoes, *Drosophila,* and Lepidoptera. These characters vary in a parallel fashion among the members of the groups.

2. The characters shown to be associated are various qualities of (A) development rate, (B) activity of adults, (C) mating habits, (D) ecological distribution, (E) coloration, (F) larval food tolerance, (G) salt tolerance, (H) adult feeding preference, and (I) morphological characteristics. The physiological characters mentioned (A–H) seem to be related to speed of body metabolism. The morphological characteristics (I) are concerned with body proportions and extent of manifestation of hair, chaetae, and spine characters.

3. The parallel variation exhibited by the several taxonomic groups is of the following sort: if the development rate is rapid, the adult activity is great, the adults will mate in small space, the insect prefers a hotter and dryer environment in which to live, its colors are lighter, the larval food and salt solution tolerances are greater, the adults (in mosquitoes) will feed on man, and there is an increase in extent of manifestation of the particular morphological characters concerned.

4. Many of the associations and parallel variations seem to be pleiotropic (manifold) effects in development. The genes concerned are probably metabolic rate genes, each having pleiotropic effects and together having an additive influence on each character. It appears probable that one or more of the associated characters are advantageous to a given form in its specific environment, though no direct evidence of selection is now available for most

of these characters. The other associated characters then may either be evolved in a parallel fashion due to parallel selection or they may be pleiotropic effects.

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CONTRIBUTIONS FROM THE LABORATORY OF VERTEBRATE BIOLOGY

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A WEIGHTING SYSTEM FOR THE ESTIMATION OF GENE FREQUENCIES FROM FAMILY RECORDS

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INTRODUCTION

IN estimating population gene frequencies a statistical complication arises when related individuals are included in the sample. This problem, recently discussed by Fisher (1940) in relation to recessive characteristics, can be referred to a fundamental consequence of heredity. Since related individuals share, in part, the same genes—same in the sense of common origin as well as like in phenotypic effect—it follows that a given gene may be counted repeatedly if all individuals are treated as though unrelated. Consequently, an estimate of gene frequency derived from a sample of n related individuals will not have the same precision as one established upon n unrelated cases, and the standard error will be underestimated if one employs the ordinary formula appropriate to a series of n independent observations.

In statistical studies on the blood groups and other inherited characters it is probable that gene frequency estimates have been computed from time to time without regard to the loss of precision resulting from the inclusion of related material. In the extreme case where the 2 relatives are monozygous twins, it is likely that such pairs would always be counted as a single individual, or, in the case of genes lacking dominance, as an observation of only 2 genes. On the other hand, pairs of parent and child, who must always share 1 gene at each locus, would customarily be counted as 2 individuals or 4 genes, rather than 3 or some smaller number.

The importance of this loss of information due to relationship will be appreciated in connection with tests of significance. Let us suppose that an investigator, having prepared antisera for testing the M and N agglutinogens, finds, among the first 4 families tested, 1 family having both parents and 4 children of the genotype MM and 3 families of the sort (MM × MN) with a total of 6 MM and 6 MN children. When all individuals are counted as unrelated, the proportion of N genes is 9/48. If it is now desired to compare this with the proportion 40/100, found by another investigator in a sample of 50 unrelated persons, the value of chi-square is $\chi^2 = 7.26$. The difference in gene frequency would, therefore, be judged significant at the 0.01 probability level, and one might suspect a true differentiation of the populations or possible errors in serological procedure. Actually, however, when the true ratio is approximately 1:2:1, the finding of 15 MM, 9 MN, and 0 NN is not especially improbable if these individuals are contained in only 4 families. For, when the children of these families are discounted on the ground that they possess no genes not already counted in the parents, the proportion of N genes is taken as 3/16, a value which, being based on only 16 genes, does not differ significantly from 40/100 ($\chi^2 = 2.67$). If the second sample had also contained relatives, the significance of the difference would have been exaggerated even more by a failure to consider relationships.

The method just used, namely that of counting only the parents, is suitable only for whole families tested for genes lacking dominance. It cannot be applied without sacrificing information when one or neither parent has been tested, even in the simplest case of 2 genes without dominance. In the case of genes showing dominance it is in no case appropriate, since, even when both parents are recorded, the children may provide further information about the parental genotypes. Fisher (1940) has outlined 2 general procedures for determining the proportion of recessives, or of recessive genes, when the data contain relatives. Both of his scoring systems are derived by the maximum likelihood method of estimation and are wholly efficient for all types of relationship. At present, however, numerical tables of the scores and weights have been made available only for dominant characteristics in pairs of parent and child, half sibs, and full sibs.

It is the purpose of the present paper to examine a simpler procedure, namely that of assigning a fixed weight to each gene or individual after all individuals have been counted as though unrelated, the weight varying only with the type of relationship exhibited. The method is therefore similar to Fisher's "fixed weight" system, in that each related group is given a score and a weight. In the present system, however, the score bears the same ratio to the weight as is shown by the unweighted numbers of genes or individuals in a direct enumeration, whereas no such simple proportionality exists between the likelihood scores and weights. This feature obviates the necessity of tabulating scores, thereby greatly reducing the number of numerical tables required, especially for large families. It further simplifies calculation by permitting the pooling of unweighted frequencies for all families or units of a given type, irrespective of their phenotypic composition.

This method, although always consistent, is generally inefficient and will, therefore, yield standard errors somewhat larger than those which could be obtained by means of likelihood scores and weights. The seriousness of this reduction in precision is naturally less in some situations than in others. In general, however, the direct weighting system should be regarded merely as a provisional method and should be discarded whenever numerical tables for a more efficient scoring system become available. Even then, the simpler method may be useful in forming a trial estimate in those cases in which the likelihood method requires an approximation procedure. An examination of the direct weighting method will also be of interest in showing to what extent the precision of gene ratios may be exaggerated when the same equations of estimation are employed for both related and unrelated material.

GENES WITHOUT DOMINANCE

In the case of 2 alleles without dominance, as exemplified by the M–N blood types, all 3 genotypes are phenotypically distinguishable. If a sample of k unrelated individuals contains a MM, b MN, and c NN, the proportion of N genes is taken as

$$q = \frac{b+2c}{2k} = \frac{x}{y}, \tag{1}$$

and the amount of information respecting q, being the reciprocal of the sampling variance, is

$$i(q) = \frac{2k}{q(1-q)}. \tag{2}$$

When the k individuals include relatives, the same equation of estimation (1) will provide a consistent estimate of the population gene proportion, even though all individuals are counted as unrelated. Owing to relationship, however, the amount of information is reduced to

$$i(q) = w\,\frac{2k}{q(1-q)}, \tag{3}$$

where w is a fraction always less than unity. Having determined the value of w for a given type of relationship, one may apply it to both the numerator and denominator of (1), obtaining scores (wx) and weights (wy) which may be conveniently summed for various groups of related or unrelated persons. The combined estimate of q for any body of genetical data will therefore be

$$q = \frac{S\{q \cdot i(q)\}}{S\{i(q)\}} = \frac{S(wx)}{S(wy)}, \tag{4}$$

and its estimated variance will be given by

$$V(q) = \frac{q(1-q)}{S(wy)}. \tag{5}$$

If there are n sets of relatives of a given type of relationship, each set containing k individuals, the variance of $q = x/2k$ may be written as

$$V(q) = \frac{1}{n} S \left\{ \frac{x}{2k} - \frac{\bar{x}}{2k} \right\}^2.$$

Hence, if P is the probability of a set containing x genes of the sort N, the expected variance is

$$V(q) = \frac{1}{4k^2n}\{S(Px^2) - S^2(Px)\},$$

or, since $q = S(Px)/2k$, because of the property of consistency,

$$V(q) = \frac{1}{4k^2n}\{S(Px^2) - 4k^2q^2\}. \tag{6}$$

Thus, the information furnished by a single set of k relatives when all individuals are counted as unrelated is

$$i(q) = \frac{4k^2}{S(Px^2) - 4k^2q^2}, \tag{7}$$

and the weight to be assigned each gene in such an enumeration is

$$w = i(q) \cdot \frac{q(1-q)}{2k} = \frac{2kq(1-q)}{S(Px^2) - 4k^2q^2}. \tag{8}$$

The procedure may be illustrated first for the case of n unrelated individuals (Table I). Here $k = 1$, and the probabilities of the three genotypes, assuming genetic equilibrium, are $(1-q)^2$, $2q(1-q)$, and q^2 for MM, MN, and NN, respectively.

TABLE I

Genotype	N Genes x	Total Genes $2k$	Probability P	Px^2
MM	0	2	$(1-q)^2$	....
MN	1	2	$2q(1-q)$	$2q - 2q^2$
NN	2	2	q^2	$4q^2$
				$2q + 2q^2$

Equation (6) leads to the familiar formula for the variance of a proportion established upon $2n$ independent observations:

$$V(q) = \frac{2q + 2q^2 - 4q^2}{4n} = \frac{q(1-q)}{2n}.$$

The information per individual in an unrelated sample is therefore $2/q(1-q)$, and the weight per gene is $i(q)q(1-q)/2k = 1$.

We may now apply the same procedure to the case of parent-child pairs, the results being shown in Table II.

TABLE II

Parent-Child Pair	x	$2k$	P	Px^2
MM, MM	0	4	$(1-q)^3$	....
MM, MN	1	4	$2q(1-q)^2$	$2q - 4q^2 + 2q^3$
MN, MN	2	4	$q(1-q)$	$4q - 4q^2$
MN, NN	3	4	$2q^2(1-q)$	$18q^2 - 18q^3$
NN, NN	4	4	q^3	$16q^3$
				$6q + 10q^2$

The variance of q determined from n pairs of parent and child is thus

$$V(q) = \frac{6q + 10q^2 - 16q^2}{16n} = \frac{3q(1-q)}{8n},$$

and $i(q) = 8/3q(1-q)$. Hence, each gene counted in parent and child will be evaluated as

$$w = \frac{q(1-q)}{4} \cdot \frac{8}{3q(1-q)} = \frac{2}{3},$$

or the entire parent-child pair as $4w = 2\frac{2}{3}$ genes.

Since every parent-child pair is certain to share only 1 gene at each locus, it may be wondered why such pairs do not tend to count as 3 genes instead of $2\frac{2}{3}$. The explanation is found in the occurrence of (MN, MN) pairs. For all other pairs the exact number of M and N genes can be specified; for example, a pair of the sort (MN, NN) contains definitely 1 M and 2 N genes. In (MN, MN) pairs, however, it is only certain that at least 1 M and 1 N gene is represented; the third gene, although certainly present, cannot be identified. Hence, such pairs, comprising an expected fraction $q(1-q)$ of all pairs, are logically worth only 2 genes, while the remainder are worth 3. This suggests that an estimate of q could be derived from parent-child pairs for which the quantity of information would be

$$[q(1-q) \cdot 2 + \{1 - q(1-q)\} \cdot 3] \frac{1}{q(1-q)} = \frac{3 - q(1-q)}{q(1-q)},$$

and such is, in fact, the amount of information which would be afforded by maximum likelihood scores when applied to parent-child pairs in the case of genes lacking dominance. Such scores, being fully efficient, utilize the whole of the information inherent in the data, which is defined (Fisher, 1935) by

$$I(q) = S\left\{\frac{1}{P}\left(\frac{dP}{dq}\right)^2\right\},$$

where *P*, as before, stands for the probability of any class. The determination of this quantity for parent-child pairs is shown in Table III.

TABLE III

Parent-Child Pair	P	dP/dq	$\frac{1}{P}\left(\frac{dP}{dq}\right)^2 \cdot q(1-q)$
MM, MM	$(1-q)^3$	$-3(1-q)^2$	$9q - 18q^2 + 9q^3$
MM, MN	$2q(1-q)^2$	$2(1-q)(1-3q)$	$2 - 14q + 30q^2 - 18q^3$
MN, MN	$q(1-q)$	$1-2q$	$1 - 4q + 4q^2$
MN, NN	$2q^2(1-q)$	$2q(2-3q)$	$8q - 24q^2 + 18q^3$
NN, NN	q^3	$3q^2$	$9q^2 - 9q^3$
			$3 - q + q^2$

$$I(q) = \frac{3 - q(1-q)}{q(1-q)}.$$

The relative efficiency of the direct weighting system for genes without dominance in parent-child pairs is therefore the ratio,

$$E = \frac{i(q)}{I(q)} = \frac{8}{3q(1-q)} \div \frac{3 - q(1-q)}{q(1-q)} = \frac{8}{9 - 3q(1-q)}.$$

TABLE IV

PROBABILITIES AND UNWEIGHTED SCORES FOR FAMILIES OF VARIOUS COMPOSITION

Father	Mother	Children	Probability, P	Genes without Dominance Number of N genes, x			Genes with Dominance Number of NN Individuals, x		
				Neither Parent Tested	One Parent Tested	Both Parents Tested	Neither Parent Tested	One Parent Tested	Both Parents Tested
MM	MM	s MM	$(1-q)^4$	0	0	0	0	0	0
MM	MN	$s-u$ MM u MN	$2q(1-q)^3C_u^s(\frac{1}{2})^s$	u	u	$u+1$	0	0	0
MM	NN	s MN	$q^2(1-q)^2$	s	s	$s+2$	0	0	1
MN	MM	$s-u$ MM u MN	$2q(1-q)^3C_u^s(\frac{1}{2})^s$	u	$u+1$	$u+1$	0	0	0
MN	MN	$s-u-v$ MM u MN v NN	$4q^2(1-q)^2C_{uv}^s(\frac{1}{4})^{s-u}(\frac{1}{2})^u$	$u+2v$	$u+2v+1$	$u+2v+2$	v	v	v
MN	NN	$s-u$ MN u NN	$2q^3(1-q)C_u^s(\frac{1}{2})^s$	$s+u$	$s+u+1$	$s+u+3$	u	u	$u+1$
NN	MM	s MN	$q^2(1-q)^2$	s	$s+2$	$s+2$	0	1	1
NN	MN	$s-u$ MN u NN	$2q^3(1-q)C_u^s(\frac{1}{2})^s$	$s+u$	$s+u+2$	$s+u+3$	u	$u+1$	$u+1$
NN	NN	s NN	q^4	$2s$	$2s+2$	$2s+4$	s	$s+1$	$s+2$

This quantity is plotted in Figure 1, which shows that the direct method has a maximum efficiency of 96.97 per cent when the 2 genes M and N are equally frequent and that the efficiency falls to 8/9 as either gene becomes very rare.

Families of s Children—Neither Parent Tested

Probably the most common unit of genetical study in human investigations is the single family, that is, a sibship having neither, one, or both parents tested. Table IV lists the various compositions of families of s children tested with respect to 2 allelic genes, M and N. It also gives the probabilities and unweighted scores, x, the latter being simply the numbers of N genes per family when all genes are counted as though unrelated. For families having neither parent tested, the table shows that the uncorrected sum of squares is

$$S(Px^2) = 4q(1-q)^3(\tfrac{1}{2})^s\Sigma\{C_u{}^s u^2\} + 4q^2(1-q)^2\Sigma\{C_{uv}{}^s(\tfrac{1}{4})^{s-u}(\tfrac{1}{2})^u(u+2v)^2\} + 2q^2(1-q)^2s^2 + 4q^3(1-q)(\tfrac{1}{2})^s\Sigma\{C_u{}^s(s+u)^2\} + 4q^4s^2,$$

where Σ stands for summation by integers from $u = 0$ to $u = s$ and from $v = 0$ to $v = s$, and where

$$C_u{}^s = \frac{s!}{(s-u)!u!} \text{ and } C_{uv}{}^s = \frac{s!}{(s-u-v)!u!v!}.$$

This reduces to

$$S(Px^2) = q(1-q)^3s(s+1) + q^2(1-q)^2(4s^2+2s) + 2q^2(1-q)^2s^2 + q^3(1-q)(9s^2+s) + 4q^4s^2 = qs(s+1) + q^2s(3s-1).$$

Since the total number of genes per sibship is $2k = 2s$,

$$S(Px^2) - 4k^2q^2 = q(1-q)s(s+1);$$

hence

$$V(q) = \frac{1}{4k^2n}\{S(Px^2) - 4k^2q^2\} = \frac{q(1-q)(s+1)}{4ns},$$

$$i(q) = \frac{4s}{q(1-q)(s+1)},$$

and

$$w = i(q)\frac{q(1-q)}{2k} = \frac{2}{s+1}. \tag{9}$$

The weight per gene in sibships of s without recorded parents is thus a function of s alone, being independent of the parametric gene proportion, q. This will be found to hold true generally for the simple weighting system when applied to data on genes lacking dominance, although the same is not true for the likelihood scores and weights. When $s = 1$, the weight (9) becomes $w = 1$, since one is then dealing with single unrelated individuals. For sib-pairs, $s = 2$ and $w = \frac{2}{3}$, which is the same as the weight found for parent-child pairs. However, the information furnished by sib-pairs is, in this instance, less than that found for parent-child pairs, and amounts to

$$I(q) = \frac{18 + 15q - 3q^2 - 24q^3 + 12q^4}{3q(1-q^2)(2-q)(1+q-q^2)}.$$

The efficiency of the simple scoring method for sib-pairs is therefore

$$E = \frac{i(q)}{I(q)} = \frac{8(1+q-q^2)(2+q-q^2)}{18+15q-3q^2-24q^3+12q^4},$$

a quantity, which, though generally greater than the corresponding value for parent-child pairs, takes the same limit (8/9) as q approaches 0 or 1 (Fig. 1). Numerical values of the weight (9) are given in Table VIII.

Families of s Children—One Parent Tested

For families having 1 parent and s children recorded, the uncorrected sum of squares is found from Table IV to be

$$\begin{aligned} S(Px^2) &= 2q(1-q)^3(\tfrac{1}{2})^s\Sigma\{C_u^s u^2\} + 2q(1-q)^3(\tfrac{1}{2})^s\Sigma\{C_u^s(u+1)^2\} + q^2(1-q)^2 s^2 \\ &\quad + q^2(1-q)^2(s+2)^2 + 4q^2(1-q)^2\Sigma\{C_{uv}^s(\tfrac{1}{4})^{s-u}(\tfrac{1}{2})^u(u+2v+1)^2\} \\ &\quad + 2q^3(1-q)(\tfrac{1}{2})^s\Sigma\{C_u^s(s+u+1)^2\} + 2q^3(1-q)(\tfrac{1}{2})^s\Sigma\{C_u^s(s+u+2)^2\} \\ &\quad + 4q^4(s+1)^2 \\ &= \tfrac{1}{2}q(1-q)^3 s(s+1) + \tfrac{1}{2}q(1-q)^3(s+1)(s+4) \\ &\quad + q^2(1-q)s + q^2(1-q)^2(s+2)^2 \\ &\quad + 2q^2(1-q)^2(2s^2+5s+2) + \tfrac{1}{2}q^3(1-q)(9s^2+13s+4) \\ &\quad + \tfrac{1}{2}q^3(1-q)(9s^2+25s+16) + 4q^4(s+1)^2 \\ &= q(s+1)(s+2) + q^2(s+1)(3s+2). \end{aligned}$$

Here $k = s+1$; hence,

$$S(Px^2) - 4k^2q^2 = q(1-q)(s+1)(s+2),$$

$$i(q) = \frac{4(s+1)}{q(1-q)(s+2)},$$

and

$$w = \frac{2}{s+2}. \tag{10}$$

The weight for a family with 1 parent and s children is thus identical with the weight for a sibship of $(s+1)$ members without recorded parents. A single tested parent counts the same as an additional sib (cf. Table VIII).

Families of s Children—Both Parents Tested

For families having both parents recorded, Table IV shows that

$$\begin{aligned} S(Px^2) &= 4q(1-q)^3(\tfrac{1}{2})^s\Sigma\{C_u^s(u+1)^2\} \\ &\quad + 4q^2(1-q)^2\Sigma\{C_{uv}^s(\tfrac{1}{4})^{s-u}(\tfrac{1}{2})^u(u+2v+2)^2\} + 2q^2(1-q)^2(s+2)^2 \\ &\quad + 4q^3(1-q)(\tfrac{1}{2})^s\Sigma\{C_u^s(s+u+3)^2\} + q^4(2s+4)^2 \\ &= q(1-q)^3(s+1)(s+4) + 2q^2(1-q)^2(2s^2+9s+8) \\ &\quad + 2q^2(1-q)^2(s+2)^2 + q^3(1-q)(9s^2+37s+36) \\ &\quad + q^4(2s+4)^2. \end{aligned}$$

The number of genes observed per family is $2k = 2s+4$, so that

$$S(Px^2) - 4k^2q^2 = q(1-q)(s+1)(s+4),$$

$$i(q) = \frac{4(s+2)^2}{q(1-q)(s+1)(s+4)},$$

and

$$w = \frac{2(s+2)}{(s+1)(s+4)}. \tag{11}$$

The weight to be assigned the entire family when both parents and s children are counted is

$$2kw = \frac{4(s+2)^2}{(s+1)(s+4)}.$$

Now, when $s = 0$, this is equal to 4, since the "family" then consists of 2 unrelated parents. For all positive values of s, however, the weight per family is less than 4, being minimum when there are 2 recorded children. For example, with $s = 1, 2, 3, 4, 5, 6$, one has $2kw = 3.6000$, 3.5556, 3.5714, 3.6000, 3.6296, 3.6571, etc., tending to 4 as s increases indefinitely.

One observes the somewhat curious fact that the inclusion of the children of 2 unrelated parents reduces the precision of the gene frequency estimate provided by the parents alone, but this appears entirely logical when it is remembered that the genotypes of the parents are completely specified by their phenotypes in the case of genes lacking dominance. The children, therefore, add nothing to the precision of the estimate, but actually detract from it, owing to chance deviations between the ratios of M and N genes in the 2 generations, such deviation tending to disappear only when an infinite sample of the parents' gametes has been obtained. When the parental genotypes are known, the probabilities of the various assortments of children are no longer functions of the population gene ratio, so that the maximum likelihood weight is always 4. To ignore the children in such families would be to substitute an efficient method, and there would seem to be no objection to such a departure from the usual procedure since the direct weighting system will necessarily have a varying efficiency for different groups of relatives.

Pairs of Relatives of any Kind or Degree

Genetical data may occasionally contain pairs of relatives other than full sibs or parent and child, such as half sibs, aunt-nephew, first cousins, and so on. For the weighting of such pairs one may now consider the information supplied by any pair of relatives whose coefficient of relationship (Wright, 1921) is r, and who are both members of a regular pedigree, i.e., one lacking inbreeding.

Let ψ_0, ψ_1, ψ_1, ψ_2 be the probabilities that an individual will share neither, the one, the other, or both genes at a given locus in a relative of degree r. Then, for any kind of relationship in regular pedigrees, $\psi_1 + \psi_2 = r$. The probabilities of the various genotype combinations in pairs of relatives may now be expressed as follows:

$$\left.\begin{aligned}
(\mathrm{MM, MM}) &= (1-q)^2\{\psi_2 + 2\psi_1(1-q) + \psi_0(1-q)^2\} \\
(\mathrm{MM, MN}) &= 4q(1-q)^2\{\psi_1 + \psi_0(1-q)\} \\
(\mathrm{MM, NN}) &= 2(1-q)^2\psi_0 q^2 \\
(\mathrm{MN, MN}) &= 2q(1-q)\{\psi_2 + \psi_1 + 2\psi_0 q(1-q)\} \\
(\mathrm{MN, NN}) &= 4q^2(1-q)\{\psi_1 + \psi_0 q\} \\
(\mathrm{NN, NN}) &= q^2\{\psi_2 + 2\psi_1 q + \psi_0 q^2\}
\end{aligned}\right\} \qquad (12)$$

Multiplying these successively by the squares of the unweighted numbers of N genes, which are $x^2 = 0, 1, 4, 4, 9, 16$, respectively, and summing,

$$S(Px^2) = q(8\psi_2 + 12\psi_1 + 4\psi_0) - q^2(8\psi_2 + 20\psi_1 + 12\psi_0).$$

Putting $\psi_2 + 2\psi_1 + \psi_0 = 1$, and subtracting $4k^2q^2 = 16q^2$, gives

$$S(Px^2) - 4k^2q^2 = 4q(1-q)(1+\psi_1+\psi_2) = 4q(1-q)(1+r).$$

Hence

$$V(q) = \frac{1}{4k^2n}\{S(Px^2) - 4k^2q^2\} = \frac{q(1-q)(1+r)}{4n},$$

$$i(q) = \frac{4}{q(1-q)(1+r)},$$

and

$$w = \frac{1}{1+r}. \tag{13}$$

For full sibs and parent-child pairs ($r = \frac{1}{2}$) the weight per gene is $w = 2/3$, as noted above; for monozygous twins ($r = 1$), $w = 1/2$; for half sibs ($r = \frac{1}{4}$), $w = 4/5$; for first cousins ($r = \frac{1}{8}$), $w = 8/9$; etc.

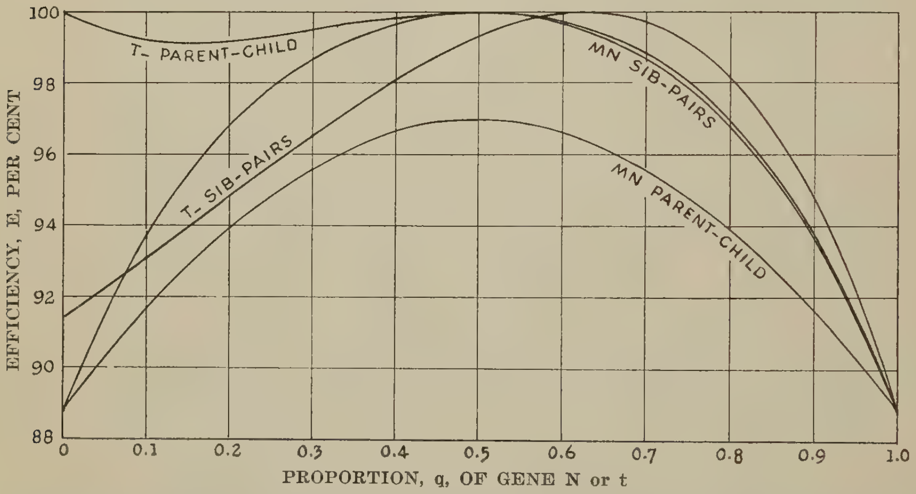

FIG. 1. Efficiency of the direct weighting of sib-pairs and parent-child pairs in estimating the gene ratio for characters showing dominance (T_) or lacking dominance (MN).

GENES WITH DOMINANCE

In the case of two alleles showing dominance, such as the "secretor" and "nonsecretor" genes in man, it is more convenient to estimate the proportion of recessives q^2, from which the gene frequency q can then readily be obtained. If a sample of k unrelated individuals contains a dominants (T_) and b recessives (tt), the square of the recessive gene proportion is estimated as

$$q^2 = \frac{b}{k}, \tag{14}$$

and the amount of information respecting q^2 is

$$i(q^2) = \frac{k}{q^2(1-q^2)}. \tag{15}$$

As in the case of genes lacking dominance, one may determine, for any group of relatives, a weight

$$w = \frac{q^2(1-q^2)}{k} \cdot i(q^2), \tag{16}$$

which, in this case, represents the weight per individual, so that the combined estimate derived from various groups of related or unrelated individuals will be

$$q^2 = \frac{S(wb)}{S(wk)}, \tag{17}$$

with variance

$$V(q^2) = \frac{q^2(1-q^2)}{S(wk)}. \tag{18}$$

The amount of information respecting the gene proportion, q, is

$$i(q) = \left(\frac{dq^2}{dq}\right)^2 \cdot i(q^2) = \frac{4S(wk)}{1-q^2}; \tag{19}$$

hence q will be estimated as

$$q = \sqrt{\frac{S(wb)}{S(wk)}}, \tag{20}$$

with variance

$$V(q) = \frac{1-q^2}{4S(wk)}. \tag{21}$$

By analogy with (7) and (8), the information and weight per individual in sets of k relatives of a given class may be written:

$$i(q^2) = \frac{k^2}{S(Px^2) - k^2q^4} \tag{22}$$

and

$$w = \frac{kq^2(1-q^2)}{S(Px^2) - k^2q^4}. \tag{23}$$

Families of s Children—Neither Parent Tested

The probabilities of the various compositions of families of s children may be obtained from Table IV by combining MM and MN to represent dominants and NN recessives. Multiplying these probabilities by the squares of the corresponding numbers of recessives, x^2, one obtains, for the case of sibships without tested parents:

$$\begin{aligned} S(Px^2) &= 4q^2(1-q)^2\Sigma\{C_v^s(\tfrac{3}{4})^{s-v}(\tfrac{1}{4})^v v^2\} + 4q^3(1-q)(\tfrac{1}{2})^s\Sigma\{C_u^s u^2\} + q^4s^2 \\ &= \tfrac{1}{4}q^2(1-q)^2 s(s+3) + q^3(1-q)s(s+1) + q^4s^2 \end{aligned}$$

$$S(Px^2) - k^2q^4 = S(Px^2) - q^4s^2 = q^2(1-q)s\{s+3+q(3s+1)\};$$

hence,

$$i(q^2) = \frac{4s}{q^2(1-q)\{s+3+q(3s+1)\}}$$

and

$$w = \frac{4(1+q)}{s+3+q(3s+1)}. \tag{24}$$

When $s = 1$, the weight becomes $w = 1$, as expected, but when $s \geqq 2$, the weight is always a function of the unknown gene proportion q, so that practical computation requires a process of interpolation with trial estimates, as is also true in the use of likelihood scores and weights. For sib-pairs ($s=2$), the information utilized by the direct weighting system is

$$i(q^2)=\frac{8}{q^2(1-q)(5+7q)},$$

whereas the total information available is, as shown by Fisher (1940),

$$I(q^2)=\frac{21-5q-8q^2+4q^3}{q^2(1-q)(3+q)(4+4q-3q^2-q^3)};$$

hence, the efficiency is, in this case,

$$E=\frac{i(q^2)}{I(q^2)}=\frac{8(3+q)(4+4q-3q^2-q^3)}{(5+7q)(21-5q-8q^2+4q^3)}.$$

This quantity is plotted in Figure 1, where it is seen that a maximum of 100 per cent efficiency is reached when $q=0.6288$ or when the percentage of recessives is 39.54. At this gene ratio the weight assigned to each individual in the sib-pair,

$$w=\frac{4+4q}{5+7q}, \tag{25}$$

becomes identical with that in the likelihood scoring system.

Numerical values of the weight (24) are given in Table IX for sibships containing 2 to 15 members.

Families of s Children—One Parent Tested

For families with 1 parent and s children tested, one obtains from Table IV:

$$\begin{aligned}S(Px^2)&=4q^2(1-q)^2\Sigma\{C_v{}^s(\tfrac{3}{4})^{s-v}(\tfrac{1}{4})^v v^2\}\\&\quad+q^2(1-q)^2+2q^3(1-q)(\tfrac{1}{2})^s\Sigma\{C_u{}^s u^2\}\\&\quad+2q^3(1-q)(\tfrac{1}{2})^s\Sigma\{C_u{}^s(u+1)^2\}+q^4(s+1)^2\\&=\tfrac{1}{2}q^2(1-q)^2s(s+3)+q^2(1-q)^2+\tfrac{1}{2}q^3(1-q)s(s+1)\\&\quad+\tfrac{1}{2}q^3(1-q)(s+1)(s+4)+q^4(s+1)^2.\end{aligned}$$

Subtracting $k^2q^4=q^4(s+1)^2$, one has

$$S(Px^2)-k^2q^4=\tfrac{1}{4}q^2(1-q)\{s^2+3s+4+q(3s^2+9s+4)\},$$

$$i(q^2)=\frac{4(s+1)^2}{q^2(1-q)\{(1+3q)s(s+3)+4(1+q)\}},$$

and

$$w=\frac{4(s+1)(1+q)}{(1+3q)s(s+3)+4(1+q)}. \tag{26}$$

For parent-child pairs ($s=1$) this reduces to

$$w=\frac{1+q}{1+2q}, \tag{27}$$

which differs from the weight for sib-pairs (25). In fact, the weight for a single parent and s children (26) is generally greater than that for a sibship of ($s+1$) without recorded parents (24), although the 2 weights tend to become identical as q approaches unity (compare Tables IX and X). This is unlike the situation found for the case of genes without dominance.

The information which parent-child pairs are capable of supplying is given by Fisher (1940):

$$I(q^2) = \frac{8 + 9q - 12q^2 + 4q^3}{4q^2(1-q)(1+2q)(1+q-q^2)},$$

whence the efficiency of using direct scores and weights for such pairs is

$$E = \frac{i(q^2)}{I(q^2)} = \frac{8(1+q-q^2)}{8+9q-12q^2+4q^3}.$$

This quantity (Fig. 1) reaches 100 per cent at the gene proportions, $q = 0$ and $q = \frac{1}{2}$, at which points the parent and child count as $2w = 2$ or 1.5 individuals, respectively.

Families of s Children—Both Parents Tested

For families having both parents and s children recorded, Table IV gives

$$S(Px^2) = 2q^2(1-q)^2 + 4q^2(1-q)^2\Sigma\{C_v^s(\tfrac{3}{4})^{s-v}(\tfrac{1}{4})^v v^2\}$$
$$+ 4q^3(1-q)(\tfrac{1}{2})^s\Sigma\{C_u^s(u+1)^2\} + q^4(s+2)^2$$
$$= 2q^2(1-q)^2 + \tfrac{1}{4}q^2(1-q)^2 s(s+3) + q^3(1-q)(s+1)(s+4) + q^4(s+2)^2,$$
$$S(Px^2) - k^2q^4 = S(Px^2) - q^4(s+2)^2 = \tfrac{1}{4}q^2(1-q)\{s^2+3s+8+q(3s^2+17s+8)\},$$
$$i(q^2) = \frac{4(s+2)^2}{q^2(1-q)\{s^2+3s+8+q(3s^2+17s+8)\}},$$
$$w = \frac{4(s+2)(1+q)}{s^2+3s+8+q(3s^2+17s+8)}. \qquad (28)$$

Numerical values of this weight are given in Table XI. Multiplying it by $(s+2)$, one has the weight for the entire family,

$$(s+2)w = \frac{4(s+2)^2(1+q)}{s^2+3s+8+q(3s^2+17s+8)}.$$

When $s = 0$, this reduces to 2; however, with $s \geqq 1$, the weight is not always equal to or greater than 2. At the limit for rare recessive genes $(q \rightarrow 0)$, the family tends to count as $4(s+2)^2/(s^2+3s+8)$ individuals; putting $s = 1, 2, 3, 4, 5, \ldots$, this becomes 3.0000, 3.5556, 3.8462, 4.0000, 4.0833, . . . , tending to 4 after reaching a maximum of 4.1739 at $s = 10$. At the other extreme, where dominants are very rare $(q \rightarrow 1)$, the weight per family becomes $2(s+2)^2/(s+1)(s+4)$. This is equal to one-half the weight for complete families tested for genes lacking dominance (11), so that the last row in Table XI duplicates the last column in Table VIII. It will be recalled that, in the case of genes lacking dominance, the inclusion of the children of 2 tested parents always reduces the weight below that for the parents alone. In the case of genes showing dominance, one would generally expect the children to provide additional information concerning the parental genotypes, except at the extreme $(q \rightarrow 1)$, at which nearly all families are of the sort (tt × tt). The likelihood weight would therefore always be 2 or greater. In the direct weighting method, however, the weight falls below 2 at a point short of $q = 1$, where the additional information supplied by the children fails to compensate for the inefficient method of scoring them. This point varies with s, as is shown in Table XI, but, in general, the children will not reduce the precision of the estimate unless the recessive gene frequency exceeds 60 per cent, in which case it would again be better to abandon the direct weighting method and count only the parents.

In the limit for very large families the weight $(s+2)w$ tends to $4(1+q)/(1+3q)$, whereas the weight provided by Fisher's likelihood scoring method tends to $(6-3q+q^2)(1+q)/(1+3q)$.[*] Consequently, the efficiency of the direct weighting method in this case approaches $4/(6-3q+q^2)$, varying from $\frac{2}{3}$ to 1 as q varies from 0 to 1.

Pairs of Relatives of any Kind or Degree

Combining the probabilities given in (12), one obtains the following probabilities for the 3 possible combinations of dominants and recessives in pairs of relatives of any kind or degree:

$$\left.\begin{aligned}(\mathrm{T}\_,\mathrm{T}\_) &= \psi_0(1-q^2)^2+2\psi_1(1-2q^2+q^3)+\psi_2(1-q^2)\\ (\mathrm{T}\_,\mathrm{tt}) &= 2q^2(1-q)(1-\psi_2+\psi_0 q)\\ (\mathrm{tt},\mathrm{tt}) &= q^2(\psi_2+2\psi_1 q+\psi_0 q^2)\end{aligned}\right\}\qquad(29)$$

Multiplying these successively by 0, 1, 4, and summing,

$$S(Px^2)=2q^2(1+\psi_2+2\psi_1 q+\psi_0 q^2).$$

Putting $k=2$, one then has

$$nV(q^2)=\frac{1}{k^2}\{S(Px^2)-k^2q^4\}=\tfrac{1}{2}q^2(1+\psi_2+2\psi_1 q+\psi_0 q^2)-q^4$$

and
$$w=\frac{q^2(1-q^2)}{k}\cdot\frac{1}{nV(q^2)}=\frac{1-q^2}{1+\psi_2+2\psi_1 q+\psi_0 q^2-2q^2}.\qquad(30)$$

Here w cannot be reduced by means of the relation $\psi_1+\psi_2=r$, as was possible in the case of genes lacking dominance. It has already been seen that the weights for sib-pairs (25) and parent-child pairs (27) differ in the case of genes showing dominance, even though the coefficient of relationship is in both cases $r=\frac{1}{2}$. These 2 types of relatives are, however, the simplest examples of 2 fundamental classes of relationship, "unilineal" and "bilineal" (Cotterman, 1941), for each of which one may obtain a general formula for w in terms of r. For unilineal relatives, who possess no 2 paths of descent which are completely different in all their links, one has $\psi_2=0$, $\psi_1=r$, and $\psi_0=1-2r$; while for symmetrical bilineal relatives, who possess 2 independent paths containing equal numbers of links, one has $\psi_2=r^2$, $\psi_1=r(1-r)$, and $\psi_0=(1-r)^2$. Making these substitutions in (30), one has

$$w_1=\frac{1+q}{1+q+2rq}\ \text{for unilineal relatives,}\qquad(31)$$

$$w_2=\frac{1+q}{1+q+2rq+r^2(1-q)}\ \text{for bilineal relatives.}\qquad(32)$$

Thus, when $r=1$, we have $w_2=\frac{1}{2}$, which is the weight per individual in the case of monozygous twins; when $r=\frac{1}{2}$, w_1 gives the weight previously found for parent and child (27), and w_2 gives the weight for sib-pairs (25); when $r=\frac{1}{4}$, one has $w_1=(2+2q)/(2+3q)$ for half sibs, grandparent-child, or aunt-nephew, etc.; and $w_2=(4+4q)/(3+5q)$ for double first cousins; and so on.

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[*] The weight given by Fisher (1940, p. 166) as $(6-3q+q^2)(1-q)/(1+3q)$ is evidently a typographical error.

NUMERICAL EXAMPLES

Table V illustrates the application of the direct weighting method to data on a pair of genetic factors lacking dominance. The record consists of the M − N blood types of all subjects examined at the University of Michigan Heredity Clinic prior to December 31, 1942, exclusive of related groups larger than a single family. The data are arranged according to the type of family, i.e., to the numbers of tested parents and children. The frequencies of MM, MN, and NN are then pooled for families of each type, and the numbers of N genes and total genes are counted without regard to relationship. The weights to be assigned each gene in the aggregate for each type of family are obtained from Table VIII.

TABLE V

ESTIMATION OF GENE RATIO FOR GENES LACKING DOMINANCE (M − N BLOOD TYPES)

Number Parents Tested	Number Children s	MM a	MN b	NN c	$b+2c=$ x	$2(a+b+c)=$ y	Weight per Gene w
Unrelated		23	54	20	94	194	1.000000
2	1	20	31	18	67	138	.600000
2	2	25	42	13	68	160	.444444
2	3	17	26	7	40	100	.357143
2	4	0	5	1	7	12	.300000
2	5	2	8	4	16	28	.259259
2	6	5	3	0	3	16	.228571
2	8	4	5	1	7	20	.185185
1	1	4	10	2	14	32	.666667
1	2	8	16	6	28	60	.500000
1	3	1	6	1	8	16	.400000
1	5	4	2	0	2	12	.285714
0	2	3	15	8	31	52	.666667
0	3	1	0	2	4	6	.500000
0	4	0	4	0	4	8	.400000
Totals		117	227	83	393	854	

$S(wx) = 238.3095,\ S(wy) = 503.8740$

The estimated proportion of N genes is therefore

$$q = \frac{S(wx)}{S(wy)} = \frac{238.3095}{503.8740} = 47.295 \text{ per cent,}$$

to which is attached a variance of

$$V(q) = \frac{0.47295 \times 0.52705}{503.8740} = 0.0004947036,$$

or a standard error of 2.224 per cent.

Had all individuals in the record been treated as unrelated, without weighting, one would have obtained: $q = 393/854 = 46.019$ per cent, with standard error $\sqrt{(0.46019 \times 0.53981)/854} = 1.706$ per cent. This estimate, which is likewise consistent, agrees well with the correctly weighted proportion, but its precision has been falsely exaggerated, the standard error being only 76.7 per cent of the appropriate value.

Since it has been shown that the direct weighting of all individuals contained in families with 2 recorded parents is less efficient than the use of the parents alone, the above weighted estimate may be slightly improved by omitting the children of such families. When this is done (Table VI), the estimate is

TABLE VI

ESTIMATION OF GENE RATIO FOR GENES LACKING DOMINANCE (M – N BLOOD TYPES), EXCLUDING CHILDREN OF TWO TESTED PARENTS

Number Parents Tested	Number Children s	MM a	MN b	NN c	$b+2c=$ x	$2(a+b+c)=$ y	Weight per Gene w
Unrelated		23	54	20	94	194	1.000000
2	0	31	61	22	105	228	1.000000
1	1	4	10	2	14	32	.666667
1	2	8	16	6	28	60	.500000
1	3	1	6	1	8	16	.400000
1	5	4	2	0	2	12	.285714
0	2	3	15	8	31	52	.666667
0	3	1	0	2	4	6	.500000
0	4	0	4	0	4	8	.400000
Totals		75	168	61	290	608	

$S(wx) = 250.3714, \quad S(wy) = 524.0286$

$$q = \frac{250.3714}{524.0286} = 47.778 \text{ per cent},$$

with standard error

$$\sqrt{\frac{q(1-q)}{524.0286}} = 2.182 \text{ per cent}.$$

The estimated number of genes sampled is now 524.0286. If likelihood scores and weights had been used for all families, this number would have been slightly larger, with a resulting slight reduction in the standard error. To use an inefficient scoring system therefore slightly minimizes the significance of a deviation in gene ratio, whereas the use of no weights at all may greatly exaggerate the significance.

By consolidating the frequencies of genotypes MM and MN in Table V, a record is obtained which will serve to illustrate the application of direct weights to data on a dominant factor. The results shown in Table VII are, therefore, those which would have been obtained if only an anti-M serum had been used. Owing to the fact that the weights for families tested with respect to dominant genes are functions of the unknown gene proportion, q, one must approximate the correctly weighted estimate, using the weights found in Tables IX, X and XI at the two trial estimates, $q = 0.45$ and $q = 0.50$. With weights appropriate for $q^2 = 20.25$ and 25.00 per cent recessives, one finds

$$q^2 = \frac{57.30}{281.58} = 0.20349 \quad \text{and} \quad q^2 = \frac{56.50}{277.34} = 0.20372,$$

TABLE VII

ESTIMATION OF GENE RATIO FOR GENES WITH DOMINANCE (M – N BLOOD TYPES, WITH MM AND MN GENOTYPES ASSUMED INDISTINGUISHABLE)

Number Parents Tested	Number Children s	MM + MN a	NN b	$a+b=$ k	Weight, w, at $q=.45$	Weight, w, at $q=.50$
Unrelated		77	20	97	1.0000	1.0000
2	1	51	18	69	.7073	.6923
2	2	67	13	80	.5485	.5333
2	3	43	7	50	.4482	.4348
2	4	5	1	6	.3791	.3673
2	5	10	4	14	.3285	.3182
2	6	8	0	8	.2898	.2807
2	8	9	1	10	.2346	.2273
1	1	14	2	16	.7632	.7500
1	2	24	6	30	.5939	.5806
1	3	7	1	8	.4823	.4706
1	5	6	0	6	.3487	.3396
0	2	18	8	26	.7117	.7059
0	3	1	2	3	.5524	.5455
0	4	4	0	4	.4514	.4444
Totals		344	83	427		

At $q=.45$: $S(wb)=57.30$, $S(wk)=281.58$;
At $q=.50$: $S(wb)=56.50$, $S(wk)=277.34$.

respectively. The former is 0.00099 above the trial estimate of 0.2025, while the latter is 0.04628 below 0.2500. The linear interpolate is

$$q^2 = 0.2025 + 0.0475 \times \frac{0.00099}{0.04727} = 0.203495$$

and the interpolated value for $S(wy)$ is

$$281.58 - 4.24 \times \frac{0.00099}{0.04727} = 281.49.$$

For the estimate of the gene proportion, q, one may now take

$$q = \sqrt{0.203495} = 45.11 \text{ per cent,}$$

with a variance of

$$V(q) = \frac{1-q^2}{4S(wy)} = \frac{0.79651}{4 \times 281.49} = 0.0007074$$

or a standard error of 2.66 per cent. By comparing this with the estimate $q = 47.778 \pm 2.182$ per cent, it is seen that precision has been lost by transforming the data into dominants and recessives, as expected. The 2 estimates, however, do not differ significantly from one another, which testifies to the internal consistency of the data and acceptable fit of the genetic hypothesis.

SUMMARY

When all members of a family or other related group are counted as though unrelated, a fixed weight may be assigned each gene or individual in the aggregate, and the usual equations for estimating the population gene ratio may then be applied to the cumulated weights. The appropriate weights for genes with and without dominance have been tabulated for families of various sizes, with neither, one or both parents recorded, and for pairs of relatives of any kind. This method of estimation, although simplifying calculation and permitting of an easier extension to complex groups of relatives, is generally inefficient and should be employed only when scores and weights for the maximum likelihood solution are not available.

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TABLES OF WEIGHTS

TABLE VIII

Genes without Dominance. Weights per Gene for Sibships of s, with Neither, One, or Both Parents Tested

Number of Children, s	Neither Parent Tested $w=\frac{2}{s+1}$	One Parent Tested $w=\frac{2}{s+2}$	Both Parents Tested $w=\frac{2(s+2)}{(s+1)(s+4)}$
0	....	1.000000	1.000000
1	1.000000	.666667	.600000
2	.666667	.500000	.444444
3	.500000	.400000	.357143
4	.400000	.333333	.300000
5	.333333	.285714	.259259
6	.285714	.250000	.228571
7	.250000	.222222	.204545
8	.222222	.200000	.185185
9	.200000	.181818	.169231
10	.181818	.166667	.155844
11	.166667	.153846	.144444
12	.153846	.142857	.134615
13	.142857	.133333	.126050
14	.133333	.125000	.118519
15	.125000	.117647	.111842

TABLE IX

GENES WITH DOMINANCE. NEITHER PARENT TESTED

Sibships of s

Weight per individual, $w = 4(1+q)/\{s+3+q(3s+1)\}$

q \ s	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	.8000	.6667	.5714	.5000	.4444	.4000	.3636	.3333	.3077	.2857	.2667	.2500	.2353	.2222
.05	.7850	.6462	.5490	.4773	.4221	.3784	.3429	.3134	.2887	.2675	.2493	.2333	.2193	.2069
.10	.7719	.6286	.5301	.4583	.4037	.3607	.3259	.2973	.2733	.2529	.2353	.2200	.2066	.1947
.15	.7603	.6133	.5140	.4423	.3882	.3459	.3119	.2840	.2606	.2408	.2238	.2091	.1962	.1847
.20	.7500	.6000	.5000	.4286	.3750	.3333	.3000	.2727	.2500	.2308	.2143	.2000	.1875	.1765
.25	.7407	.5882	.4878	.4167	.3636	.3226	.2899	.2632	.2410	.2222	.2062	.1923	.1802	.1695
.30	.7324	.5778	.4771	.40625	.3537	.3133	.2811	.2549	.2332	.2149	.1992	.1857	.1739	.1635
.35	.7248	.5684	.4675	.3971	.3450	.3051	.2734	.2477	.2264	.2085	.1932	.1800	.1685	.1584
.40	.7179	.5600	.4590	.3889	.3373	.2979	.2667	.2414	.2205	.2029	.1879	.1750	.1637	.1538
.45	.7117	.5524	.4514	.3816	.3305	.2915	.2607	.2358	.2152	.1980	.1833	.1706	.1596	.1499
.50	.7059	.5455	.4444	.3750	.3243	.2857	.2553	.2308	.2105	.1935	.1791	.1667	.1558	.1463
.55	.7006	.5391	.4382	.3690	.3188	.2805	.2505	.2263	.2063	.1896	.1754	.1632	.1525	.1432
.60	.6957	.5333	.4324	.3636	.3137	.2759	.2462	.2222	.2025	.1860	.1720	.1600	.1495	.1404
.65	.6911	.5280	.4272	.3587	.3091	.2716	.2422	.2185	.1991	.1828	.1690	.1571	.1468	.1378
.70	.6869	.5231	.4224	.3542	.3049	.2677	.2386	.2152	.1960	.1799	.1663	.1545	.1444	.1355
.75	.6829	.5185	.4179	.3500	.3011	.2642	.2353	.2121	.1931	.1772	.1637	.1522	.1421	.1333
.80	.6792	.5143	.4138	.3462	.2975	.2609	.2323	.2093	.1905	.1748	.1614	.1500	.1401	.1314
.85	.6758	.5103	.4100	.3426	.2942	.2578	.2295	.2067	.1881	.1725	.1593	.1480	.1382	.1296
.90	.6726	.5067	.4064	.3393	.2912	.2550	.2269	.2043	.1858	.1704	.1574	.1462	.1364	.1279
.95	.6695	.5032	.4031	.3362	.2884	.2524	.2245	.2021	.1837	.1685	.1555	.1444	.1348	.1264
1.00	.6667	.5000	.4000	.3333	.2857	.2500	.2222	.2000	.1818	.1667	.1538	.1429	.1333	.1250

TABLE X

GENES WITH DOMINANCE. ONE PARENT TESTED

Families of s Children

Weight per individual, $w = 4(s+1)(1+q)/\{s(s+3)(1+3q)+4(1+q)\}$

q \ s	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	1.0000	.8571	.7273	.6250	.5455	.4828	.4324	.3913	.3571	.3284	.3038	.2826	.2642	.2479	.2336
.05	.9545	.8025	.6747	.5769	.5020	.4434	.3967	.3586	.3271	.3006	.2780	.2585	.2416	.2267	.2135
.10	.9167	.7586	.6331	.5392	.4681	.4129	.3690	.3333	.3039	.2791	.2581	.2399	.2242	.2103	.1981
.15	.8846	.7225	.5993	.5088	.4409	.3884	.3468	.3132	.2854	.2620	.2422	.2252	.2103	.1973	.1858
.20	.8571	.6923	.5714	.4839	.4186	.3684	.3288	.2967	.2703	.2481	.2293	.2131	.1991	.1867	.1758
.25	.8333	.6667	.5479	.4630	.4000	.3518	.3137	.2830	.2577	.2366	.2186	.2031	.1897	.1779	.1675
.30	.8125	.6446	.5279	.4452	.3842	.3377	.3010	.2715	.2471	.2268	.2095	.1947	.1818	.1705	.1606
.35	.7941	.6255	.5106	.4299	.3707	.3256	.2901	.2616	.2381	.2185	.2018	.1875	.1751	.1642	.1546
.40	.7778	.6087	.4956	.4167	.3590	.3151	.2807	.2530	.2303	.2112	.1951	.1813	.1693	.1587	.1494
.45	.7632	.5939	.4823	.4050	.3487	.3060	.2725	.2455	.2234	.2049	.1893	.1758	.1642	.1540	.1449
.50	.7500	.5806	.4706	.3947	.3396	.2979	.2652	.2389	.2174	.1994	.1841	.1711	.1597	.1498	.1410
.55	.7381	.5688	.4601	.3856	.3316	.2907	.2587	.2331	.2120	.1945	.1796	.1668	.1557	.1460	.1375
.60	.7273	.5581	.4507	.3774	.3243	.2843	.2530	.2278	.2073	.1901	.1755	.1630	.1522	.1427	.1343
.65	.7174	.5485	.4422	.3700	.3178	.2785	.2478	.2231	.2030	.1861	.1718	.1596	.1490	.1397	.1315
.70	.7083	.5397	.4345	.3632	.3119	.2732	.2431	.2189	.1991	.1825	.1685	.1565	.1461	.1370	.1289
.75	.7000	.5316	.4275	.3571	.3066	.2685	.2388	.2150	.1955	.1793	.1655	.1537	.1435	.1345	.1266
.80	.6923	.5243	.4211	.3516	.3017	.2642	.2349	.2115	.1923	.1763	.1628	.1512	.1411	.1323	.1245
.85	.6852	.5175	.4151	.3464	.2972	.2602	.2313	.2083	.1894	.1736	.1603	.1488	.1389	.1302	.1226
.90	.6786	.5112	.4097	.3417	.2931	.2565	.2281	.2053	.1866	.1711	.1579	.1467	.1369	.1283	.1208
.95	.6724	.5054	.4047	.3374	.2892	.2531	.2250	.2025	.1841	.1688	.1558	.1447	.1350	.1266	.1192
1.00	.6667	.5000	.4000	.3333	.2857	.2500	.2222	.2000	.1818	.1667	.1538	.1429	.1333	.1250	.1176

TABLE XI

GENES WITH DOMINANCE. BOTH PARENTS TESTED

Families of s Children

Weight per individual, $w = 4(s+2)(1+q)/\{s^2+3s+8+q(3s^2+17s+8)\}$

q \ s	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	1.0000	.8889	.7692	.6667	.5833	.5161	.4615	.4167	.3793	.3478	.3210	.2979	.2778	.2602	.2446
.05	.9403	.8116	.6931	.5972	.5213	.4609	.4122	.3723	.3392	.3113	.2875	.2670	.2492	.2336	.2198
.10	.8919	.7521	.6358	.5455	.4753	.4200	.3757	.3395	.3095	.2842	.2626	.2441	.2279	.2137	.2012
.15	.8519	.7050	.5913	.5055	.4399	.3886	.3476	.3142	.2865	.2632	.2434	.2263	.2114	.1983	.1868
.20	.8182	.6667	.5556	.4737	.4118	.3636	.3253	.2941	.2683	.2466	.2281	.2121	.1982	.1860	.1753
.25	.7895	.6349	.5263	.4478	.3889	.3433	.3072	.2778	.2535	.2330	.2156	.2006	.1875	.1760	.1659
.30	.7647	.6082	.5019	.4262	.3699	.3265	.2921	.2642	.2411	.2217	.2052	.1910	.1786	.1677	.1580
.35	.7431	.5854	.4813	.4081	.3539	.3124	.2795	.2528	.2308	.2123	.1965	.1829	.1710	.1606	.1514
.40	.7241	.5657	.4636	.3925	.3403	.3003	.2687	.2431	.2219	.2041	.1890	.1759	.1646	.1546	.1457
.45	.7073	.5485	.4482	.3791	.3285	.2898	.2593	.2346	.2142	.1971	.1825	.1699	.1590	.1494	.1408
.50	.6923	.5333	.4348	.3673	.3182	.2807	.2512	.2273	.2075	.1910	.1769	.1647	.1541	.1448	.1365
.55	.6788	.5199	.4229	.3570	.3091	.2727	.2440	.2208	.2017	.1856	.1719	.1601	.1498	.1408	.1328
.60	.6667	.5079	.4124	.3478	.3011	.2656	.2376	.2151	.1964	.1808	.1675	.1560	.1460	.1372	.1294
.65	.6556	.4972	.4029	.3396	.2939	.2592	.2319	.2099	.1918	.1765	.1635	.1523	.1426	.1340	.1264
.70	.6456	.4875	.3944	.3322	.2874	.2535	.2268	.2053	.1876	.1727	.1600	.1490	.1395	.1311	.1237
.75	.6364	.4786	.3867	.3256	.2816	.2483	.2222	.2011	.1838	.1692	.1568	.1461	.1367	.1285	.1212
.80	.6279	.4706	.3797	.3195	.2763	.2437	.2180	.1974	.1803	.1660	.1538	.1433	.1342	.1261	.1190
.85	.6201	.4632	.3734	.3140	.2715	.2394	.2142	.1939	.1772	.1631	.1512	.1409	.1319	.1240	.1170
.90	.6129	.4565	.3675	.3089	.2671	.2355	.2107	.1908	.1743	.1605	.1488	.1386	.1298	.1220	.1151
.95	.6062	.4502	.3621	.3043	.2630	.2319	.2075	.1879	.1717	.1581	.1465	.1365	.1278	.1202	.1134
1.00	.6000	.4444	.3571	.3000	.2593	.2286	.2045	.1852	.1692	.1558	.1444	.1346	.1261	.1185	.1118

The weights below the dotted line are less than $2/(s+2)$, so that omission of the children will provide a more efficient estimate in such cases.

CONTRIBUTIONS FROM THE LABORATORY OF VERTEBRATE BIOLOGY

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EFFECTIVENESS OF SELECTION BY OWLS OF DEER-MICE (*PEROMYSCUS MANICULATUS*) WHICH CONTRAST IN COLOR WITH THEIR BACKGROUND

BY LEE R. DICE

Accepted for publication June 20, 1946.

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INTRODUCTION

ALTHOUGH it is generally agreed by biologists that natural selection must play an important role in the evolution of protective coloration and of the other adaptations of animals, only a few measurements have been made of the actual effectiveness of selection under any particular set of conditions. Many students of evolution have in recent years come to assume a very low effectiveness for natural selection. Sewall Wright (1940: 178), for example, doubts that the mutations which are important in evolution produce a sufficiently large selective difference to be demonstrable in the laboratory.

Should the selective advantage of adaptative characters, however, be in general very low, then the resulting rate of evolution must be very slow, too slow probably to account for the evolution of our existing plants and animals during the estimated period of geologic time. Moreover, unless selection is very effective, at least at some times and in some places, there is difficulty in explaining the demonstrated instances of the rapid origin of races and species in certain localities. The only alternative to effective selection seems to be

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the hypothesis of Goldschmidt (1940) of large and successful saltations, but this hypothesis has not generally been accepted by biologists.

In order to secure a measure of the possible rate of selection under controlled conditions I began a series of laboratory experiments in 1938, using various owls as predators and deer-mice (*Peromyscus*) of various colors as prey. Some preliminary experiments were conducted in outdoor runs. It was early discovered, however, that light intensity played an important role in the activities of the deer-mice. The experiments were then moved to a reaction room, specially constructed for the study, inside the Vertebrate Biology Building on the University of Michigan campus.

In the reaction room the owls proved to be able to capture living deer-mice in complete darkness, presumably by the sense of hearing. The lower limit of illumination under which the owls could find their prey by sight was, therefore, determined by the use of dead mice (Dice, 1945). Attempts were next made to set up an experimental situation reproducing so far as possible the conditions in nature under which the owls capture their living prey. After many discouraging failures a mouse "jungle" was designed which, it is believed, simulated fairly well, but in a simplified and standardized manner, the conditions in the natural habitats of the animals. By the use of this "jungle" a measure was obtained of the tendency of the owls to select those mice which are most conspicuous in color when viewed against the soil or other objects. These experiments were completed in August, 1945.

ACKNOWLEDGMENTS

The laborious work of carrying out the experiments here reported was conducted mostly by Mary Jane Lagler. The photometric readings of pelage colors and of soil colors were made by William R. Jalosky. For suggestions in regard to the statistical treatment of the data I am indebted to C. W. Cotterman.

SELECTION INDEX

As a measure of the effectiveness of selection in any given situation I propose the use of a "selection index." Where two types of individuals are equally abundant in an experimental population and are then exposed to predation or to some other form of mortality, both types should, if there is no differential selection, be taken in equal numbers. Should one type be taken in greater numbers than the other, this will indicate that selection is operating. If a and b, respectively, are the numbers taken of the two types A and B, then the relative selection in favor of or against type A will be measured by the difference between the observed value of a and its expected value $\frac{1}{2}(a+b)$, this difference being divided by $\frac{1}{2}(a+b)$:

$$\text{Selection index} = \frac{a - \frac{1}{2}(a+b)}{\frac{1}{2}(a+b)} = \frac{a-b}{a+b}.$$

The selection index evidently may vary between +1.0 and −1.0, being 0.0 when there is an absence of selective effect.

The significance of the deviation of the selection index from 0.0 may be tested by the usual chi-square formula for a 1:1 ratio:

$$\text{Chi-square of selection index} = \frac{(a-b)^2}{a+b}.$$

The formula given above for the calculation of the selection index applies, of course, only to a situation in which the numbers of the two types of prey are equal at the time when selection occurs. To measure the selection that occurs in situations where two or more types of prey are unequal in abundance, as will often occur in nature, more complex formulas would have to be developed.

In the experiments hereinafter described, the proportional numbers of the two types of prey exposed in any trial did in fact differ as soon as any individual had been captured by the predator. This difference in the available numbers of the two types of prey would decrease the selective pressure against the type taken most frequently and would depress the selection index toward zero in all those trials in which the predator took more than a single individual. In no case, therefore, will this source of error in the calculation of the selection index invalidate the conclusions reached. On the contrary, the true selection indexes would have been slightly higher than those calculated if the numbers of the two types of prey offered to the predator could have been kept precisely equal each time he made a selection.

The selection index obviously is a measure only of the selective advantage enjoyed by a particular phenotype under the conditions specified. The selection index is, therefore, a very different statistic than the selection coefficient (Haldane, 1932: 97; Wright, 1940: 165). The selection coefficient is calculated from the changes in the gene frequencies from generation to generation in the population under study. In the experiments here described the genotypes of the animals tested were unknown and the selection coefficients cannot be calculated.

EXPERIMENTAL METHODS

In my attempts to measure the effectiveness of selection under controlled laboratory conditions, I used several species of owls as the predators, and deer-mice (*Peromyscus maniculatus*) were used as the prey. The experiments were conducted in the same reaction room in which the minimum intensities of light under which owls are able to find dead prey were determined previously (Dice, 1945). This room is divided by a low partition two feet high into two compartments, each about nine by ten feet in size. Special lamps placed over the middle of each compartment make possible the control of the light intensities. The several types of soils used on the floors of the experimental compartments either matched closely or contrasted with the pelage colors of the mice being tested.

Each ''trial'' in one of these experiments consisted of exposing four live mice of one color and four of a contrasting color together in one compartment for a fifteen-minute period. In each experiment the colors of the mice and of the soils were arranged so that one kind of mouse matched the color of the soil in one compartment and the other kind of mouse matched the color of the soil in the other compartment. The mice were exposed alternately on the two colors of soils by using first one and next the other compartment. In every experiment, therefore, each kind of mouse was exposed an equal number of times on soil which it matched in color and on soil with which it contrasted in color. Furthermore, at the beginning of each trial the owl had the choice of the same number of mice of the two pelage colors, one kind matching the color of the soil and the other contrasting with it.

The mice were kept in the same room with the owl for a number of nights before being used in an experiment. It has been our experience that a mouse under these conditions quickly becomes frightened of the owl and will attempt to keep out of its reach if possible. In all the experiments in which the "jungle" was used the door of the nest box in which the mice were kept was opened at the beginning of the experiment and the animals were free to go out under the jungle or to remain in their nest box.

The trials were nearly all carried out in the morning, after the reaction room had been illuminated during the night by the fairly bright light of six 150-watt Mazda bulbs. Each trial period lasted for fifteen minutes, during which time the mice were exposed to the owl at the specified light intensity. Then the bright lights were turned on; the owl was driven back to his box, the door of which was then closed and the remaining mice were counted. If the owl had captured one or more mice, the carcasses usually were evident. If the owl had not captured a mouse, a second trial often followed immediately, usually at a different light intensity from that of the first trial. Rarely three, and still more rarely four, trials at different light intensities were given in a single morning. After the owl had captured a mouse in a given trial, the operations were discontinued for the day. Fairly dim lights then illuminated the room until noon, when all uneaten carcasses were removed. During the afternoon the room was kept in total darkness until 6 P.M., when the bright lights were automatically turned on again by a time clock.

By this artificial reversal of the usual cycle of day and night, the owl was always hungry in the morning and the mice began to move about and to expose themselves as soon as the bright lights were turned off in the reaction room at the beginning of each trial period.

Photometric Measurements

All the color measurements for this study, both of soil color and of pelage color, were made with a photovolt reflection meter, model 610. This instrument is manufactured by the Photovolt Corporation of New York City. The color reading is made by a movable search unit which is connected with the body of the instrument by a multiple electric cord. A concentrated beam of light from a small electric bulb in the search unit is reflected from the specimen at an angle of approximately forty-five degrees to a circular photoelectric cell which surrounds the light beam. The photosensitive cell when stimulated by this light produces sufficient electric current to be read on a sensitive galvanometer. A constant-voltage transformer forms part of an instrument to prevent fluctuations in line voltage from affecting the accuracy of the readings.

The area of specimen surface measured by the reflection meter, as adapted for this study, is a rectangle 9.3 by 7.5 millimeters. These are the dimensions of an opening in a blackened metal plate which is fastened over the face of the search unit. This plate was not supplied by the manufacturer, but was added by us. The opening in the metal plate is on the face of the search unit in such a position that it passes the brightest part of the light beam.

In order to locate precisely the area on the specimen to be read by the search unit, an additional finding device is necessary. This device also was constructed by us. A two- by two-inch square, lantern-slide cover-glass was mounted on the face of a small, square, wooden frame, whose outside dimensions were the same as the diameter of the search unit. Scratches were made on the glass plate to outline an area coinciding in dimensions and in position with the opening in the metal plate on the face of the search unit. A larger wooden holding frame was also constructed with inside dimensions that allowed it to fit loosely around the outside of the finding unit. Because the finding unit has the same outside dimensions as the search unit, the holding frame also fits loosely around the outside of the search unit. The search unit, however, is round, whereas the holding frame is square. A notch is therefore provided on one side of the holding frame to receive the projecting end of the glass filter inserted into the search unit, thereby keeping the search unit always oriented in the same direction in respect to the finding unit.

The specimen to be measured is placed flat on a table with the surface to be read facing upward. The finding unit is then placed on the specimen and moved about until the scratches on the glass plate of the unit enclose the exact area desired to be read. The holding frame is next placed outside and around the finding unit. Care is taken not to move the position of this frame when the finding unit is removed. The search unit is then placed in position, replacing the finding unit, and the reading is made.

Three color filters, red, green, and blue, furnished with the instrument, were used in making the photometric readings. The total light reflected from any one of these filters and reflected from a freshly scraped, white, magnesium carbonate block was taken as 100. The needle of the galvanometer was set at zero when the light from the search unit was projected into a hollow, black-lined cylinder of paper. Each reading of the galvanometer, therefore, is actually the percentage of light of the given color reflected from the specimen as compared to the amount reflected from the standard magnesium carbonate block.

Soils

Three types of soils were used in the various experiments to cover the floors of the compartments in which the mice were released. The "silica sand" employed is a commercial product that is nearly white. The "gully soil" has a dull, slightly yellowish gray color. It was taken from a small eroded gully near the Vertebrate Biology Building. The "Ann Arbor sand" is a building sand from one of the commercial sand pits in the vicinity of Ann Arbor. This sand is a mixture containing grains of several sizes and colors. Particles of gray are most numerous, but many other particles are black. This sand is in general appearance darker and less buffy in hue than the gully soil.

The reflection meter readings of the colors of these soils given in Table I are each the mean of fifteen or more readings of air-dry soil, each reading being taken on a different spot on the sample. The soils were very uniform in color and in shade and the reflection meter readings did not vary more than about one or at most two units on either side of the mean.

TABLE I

COLORS OF SOILS USED IN THE EXPERIMENTS WITH OWLS AND DEER-MICE

Means of fifteen or more color readings.

Type of Soil	Mean Reflection Meter Readings		
	Red	Green	Blue
Silica sand	53	46	36
Gully soil	39	25	16
Ann Arbor sand	33	22	15

It will be noted (Table I) that the silica sand is much paler in color than either of the other two soils used in the experiments. This is shown by its higher readings for all color screens. The gully soil has higher readings for red and for green than has the Ann Arbor sand, but does not differ very much in the reading for blue. This indicates the more reddish buffy hue of the gully soil as contrasted with the Ann Arbor sand. In general shade the Ann Arbor sand is only slightly darker than the gully soil.

Predators

Two species of owls served as predators in these experiments: a male long-eared owl (*Asio wilsonianus*) from Pennsylvania and a male barn owl (*Tyto alba pratincola*) from southern Michigan. These were two of the birds that were used in my previous experiments on the light discrimination of owls (Dice, 1945). Both owls were fully adult.

About a fifteen-minute period seems usually to be required for an owl of either of these species to catch and completely to eat a mouse. However, an owl sometimes caught two or more rarely three mice in a fifteen-minute period, and one once took four during this length of time. When more than one mouse is captured during a trial period, the carcasses are rarely all consumed before the end of the fifteen minutes. Each mouse that was taken or injured by the owl was counted as a capture.

Prey

Four types of deer-mice of different colors were used in the various experiments as prey: "ivory," "discard gray," "buff," and "*blandus* gray." These mice represented different laboratory strains of the single species *Peromyscus maniculatus.* They were, in general, of similar size and of similar type of activity. Their ages varied considerably, but all were of fully adult size. Both males and females were represented, and no attempt was made to discriminate between the two sexes in the records of the number of captures made by the owls.

The "ivory" mice are the result of a recessive mutation which produces a pelage color just slightly more gray than the pure white of the albino strain (Huestis, 1938; Clark, 1938: 2). The ivory mutation was originally discovered by Huestis in a wild mouse of the subspecies *rubidus.* Our animals were taken from the much mixed laboratory population.

The "discard-gray" mice were a rather variable lot of discards from the breeding experiments currently being conducted in the *Peromyscus* laboratory. They were of many different genetic constitutions. Though all were gray in general hue, many different shades of gray were represented. Many of them matched fairly well the color of the gully soil, but others were somewhat darker than this soil.

The "buff" deer-mice used in one experiment (Table V) were the F_1 offspring of a cross between a highly selected strain of *Peromyscus m. blandus* from Tularosa, New Mexico, and various strains of buff mice present in the laboratory. It is probable that the buff character of these laboratory strains was mostly derived originally from the subspecies *nebrascensis,* two stocks of which were involved in their ancestry. *Exiguus, gambeli, rubidus, rufinus,* and perhaps other subspecies also contributed to the ancestry of these buff strains. The *blandus* strain used as one of the parents in the cross was highly inbred and was undoubtedly homozygous for many genes. Presumably, for this reason the buff F_1 offspring were very uniform both in pelage color and in size. Buff being dominant in heredity all the F_1 offspring were buff.

The "*blandus*-gray" mice were the F_1 offspring of a cross between highly inbred, "tipped dark gray" and "tipless gray" strains of *blandus,* both originally from Tularosa, New Mexico. In spite of the inbreeding of the two parental strains of these mice and their consequent considerable amount of homozygosity, the F_1 offspring used in the experiment exhibited moderate variability in shade of pelage. They all had a slight tinge of buffy, and their general hue closely matched that of the Ann Arbor sand, but they were not all alike in shade. On the average they matched to my eyes the shade of the Ann Arbor sand fairly well, but some individuals were paler than the Ann Arbor sand and some were darker.

It will be noted that both the "buff" and the "*blandus*-gray" mice used in the experiment reported in Table V had at least one parent of *blandus* stock from Tularosa. As a result of this similar ancestry these two types of mice were very similar in size and in amount of activity and differed, so far as could be observed, only in their pelage color.

The ivory mice were the palest in pelage of the deer-mice used in these experiments as is shown by their high reflection meter readings (Table II).

TABLE II

COLORS OF FLAT SKINS OF DEER-MICE USED IN THE EXPERIMENTS

Type of Mouse	Specimens	Reflection Meter Readings Means and Extremes		
		Red	Green	Blue
Ivory				
Dorsal stripe	5	64.40 (62–68)	49.40 (46–53)	39.00 (37–41)
Side stripe	5	70.00 (67–74)	56.00 (52–62)	45.00 (41–49)
Buff				
Dorsal stripe	15	18.20 (14–24)	8.93 (6–12)	6.80 (6– 8)
Side stripe	15	29.53 (22–35)	13.07 (10–16)	9.40 (8–11)
Blandus-gray				
Dorsal stripe	9	15.33 (13–18)	10.78 (8–13)	9.22 (6–11)
Side stripe	9	27.89 (21–31)	19.56 (14–23)	17.00 (11–20)

The buff mice averaged higher in readings for reflected red than did the *blandus*-gray mice, both on the dorsal stripe and on the side of the body. In readings for reflected green and reflected blue, on the contrary, the *blandus*-gray mice averaged the higher. This shows that the buff mice had a redder, more buffy hue than did the *blandus*-gray individuals, although the general shade of color was slightly paler in the *blandus*-gray than in the buff mice.

Because of the considerable variability in hue and in shade of the discard-gray mice, no attempt is made to present photometer readings of their pelage color. In every case, however, the discard-gray mice were much darker than the ivory mice against which they were tested.

Mouse "Jungles"

When the floor of the reaction room was bare of obstructions, the owls that served as predators in these experiments were able, as has already been mentioned, to capture living deer-mice in complete darkness. The owls presumably were able to do this through the sense of hearing. Various expedients were tried to prevent the owls from capturing the deer-mice by hearing alone and to force them to use the sense of sight. After many failures to achieve this end, I contrived a mouse "jungle" in which the owls almost never caught a mouse in complete darkness, but only when the light was of an intensity under which it had earlier been demonstrated that the owls could see and find dead mice of this species (Dice, 1945).

The mouse "jungles" were constructed of wooden sticks screwed together to form an open latticework. Sticks three-fourth by three-fourth inches in size and mostly twelve inches in length were placed vertically in a checkerboard pattern, with the sticks spaced eight inches apart on their centers. These vertical posts were connected by horizontal pieces of rough, split lath, three-eighth by three-fourth inches in cross section, which were placed three and one-half inches above the floor in one direction and four and one-half inches above the floor crosswise above the first set of sticks. The space above the floor of the reaction room was thus broken up by the "jungle" into little cells, the largest horizontal dimensions of any opening being a little less than eight by eight inches.

The sticks making up each "jungle" were painted to match the color of the soil of the compartment, so that should a mouse climb up on the sticks he would not be any more or less conspicuous than if he remained on the floor.

In the "jungle" the mice could run freely about under the latticework of horizontal sticks, the only obstructions to their progress being the vertical posts. Although the mice were exposed to attack by the owls, they were nevertheless protected to some extent, because of the handicap imposed on the predator by the presence of the sticks. An owl could reach through the meshes between the latticework formed by the sticks and could catch the mice fairly readily when the light intensity was sufficiently high for him to see his prey.

Although the owl undoubtedly could hear the mice running about under the "jungle," he evidently could only rarely secure them without the aid of sight. Observations were made of the method by which owls secure mice on an unobstructed floor: the wings usually are used to enfold the mice and draw them within reach of the talons. Presumably, this is the manner in which mice are caught in complete darkness after their general position has been located by hearing. In the "jungle," however, this method of capture could not be employed because of the presence of the sticks. The owl, accordingly, was forced to depend on sight for securing the prey. The "jungle," therefore, probably reproduced to a considerable extent the conditions in nature when the prey is moving about among such obstructions as shrubs or herbs.

RESULTS OF THE EXPERIMENTS WITH OWLS AND DEER-MICE

When ivory and discard-gray mice were exposed to the barn owl on silica sand and on gully soil on alternate nights, without any protection, either by a "jungle" or otherwise, 82 conspicuous and 70 concealingly colored mice were taken in 120 fifteen-minute trials (Table III). Approximately as many mice per period, however, were taken in complete darkness and in extremely dim light as were taken under illuminations in which the owl undoubtedly could see his prey. In darkness the owl certainly must have caught mice without using the sense of sight. The selection index against the conspicuous mice in the whole experiment is 0.079, but this is statistically not significant.

With the lack of any protection for the mice in this experiment the owl evidently was able to capture his prey by some sense other than vision. This is demonstrated by the fact that the owl was able to catch the mice in complete darkness. It may be presumed that hearing was the major sense employed to locate the prey, at least in darkness and in very dim light. The failure of the owl in this experiment to show any considerable tendency to select the more conspicuously colored mice is undoubtedly due to his lack of dependence upon sight for the capture of his prey.

TABLE III

SELECTION OF IVORY AND DISCARD-GRAY DEER-MICE BY BARN OWL

Floor of selection room bare; no protection for mice. Four live mice of each of two colors exposed in same compartment for each trial; ten trials on each soil type; fifteen-minute periods.

Illumination in Foot Candles (Approximate)	Mice Taken					Conspicuous Mice Taken	Concealingly Colored Mice Taken
	On Silica Sand		On Gully Soil		Average per Trial		
	Ivory	Discard-gray	Ivory	Discard-gray			
0.000,003,5	8	6	5	3	1.10	11	11
0.000,002,0	5	9	7	6	1.35	16	11
0.000,000,53	5	9	7	2	1.15	16	7
0.000,000,31	7	6	10	11	1.70	16	18
0.000,000,08	8	4	7	3	1.10	11	11
Dark	6	5	7	6	1.20	12	12
Total	39	39	43	31	....	82	70

When in another experiment the mice were given partial protection by placing a wooden "porch" over the entrance to the next box, there was still little indication of the selection of mice of any particular pelage color. Presumably, the owl awaited and captured whatever mouse first wandered outside the protective cover of the "porch." The color of the mouse first exposed and consequently caught was a matter of chance.

A series of experiments was then carried out with a two-foot wide "jungle" of sticks, constructed as above described, around the outside of each compartment, leaving the middle of the compartment open. Four each of ivory and of discard-gray mice were exposed to the owl for fifteen-minute periods on silica sand and gully soil on alternate days.

Under these conditions the barn owl demonstrated a striking selection of the more conspicuous mice (Table IV). The selective effect was evident at every light intensity tested, except darkness, the dim light of 0.000,000,31 foot candle, and under the relatively bright light of 0.017 foot candle. The number of mice taken per period was relatively low under the brighter intensities used in the experiments, evidently because the mice in these relatively bright lights remained inside their nest boxes and, therefore, only infrequently exposed themselves to the owl. Nevertheless, selection is indicated to be generally effective over the whole range of light intensities from 0.24 to 0.000,000,08 foot candle.

If the number of mice taken in the whole experiment is added, omitting only those taken in complete darkness, it is found that 124 conspicuously colored mice and 68 that were concealingly colored were taken. The index of selection is 0.292, and the chi-square of the deviation from a 1:1 ratio is 16.333, a highly significant amount. There is no significant difference between the numbers of discard-gray and of ivory mice taken, nor between the numbers taken on silica sand and on gully soil. Neither is there any important difference between the selection indexes on the silica sand and on the gully soil, these being respectively, 0.289 and 0.294.

TABLE IV

SELECTION OF IVORY AND DISCARD-GRAY DEER-MICE BY BARN OWL

''Jungle'' of sticks around outside of each compartment. Four live mice of each of two colors exposed in same compartment for each trial; ten trials on each soil type; fifteen-minute periods.

Illumination in Foot Candles (Approximate)	Mice Taken					Conspicuous Mice Taken	Concealingly Colored Mice Taken
	On Silica Sand		On Gully Soil		Average per Trial		
	Ivory	Discard-gray	Ivory	Discard-gray			
0.24	1	4	3	1	0.45	7	2
0.017	4	2	3	1	0.50	5	5
0.004,8	1	4	4	1	0.50	8	2
0.001,4	2	11	9	7	1.45	20	9
0.000,10	7	7	11	3	1.40	18	10
0.000,026	4	6	6	4	1.00	12	8
0.000,013	3	7	7	6	1.15	14	9
0.000,003,5	......	4	7	1	0.60	11	1
0.000,002,0	2	5	4	5	0.80	9	7
0.000,000,53	1	1	2	1	0.25	3	2
0.000,000,31	4	4	2	2	0.60	6	6
0.000,000,08	3	3	8	4	0.90	11	7
Dark	1	.....	1	2	0.20	1	3
Totals	33	58	67	38	....	125	71
Totals omitting ''dark'' ...	32	58	66	36	....	124	68

The fact that four mice were taken in darkness in twenty trials shows that in this experiment the owl was able to capture a mouse occasionally by some sense other than sight. Presumably, most or all of these mice were captured in the middle of the room, where they were not protected by the ''jungle.'' Tracks in the sand indicated that at least one mouse actually was captured in this open space.

The barn owl in this experiment was able to capture mice readily under an illumination as dim as 0.000,000,08 foot candle. He was actually very successful in this light intensity in capturing live mice (0.90 per fifteen-minute period), though in an earlier experiment he had failed to find dead mice in such very dim light (Dice, 1945 : 398). Moving objects are probably seen by the owl more readily in very dim light than are stationary ones. Furthermore, the owl can probably determine the general location of a live mouse by the sounds it produces in moving about. These considerations may explain why this owl was able to secure live mice in such very weak light, whereas he had previously failed to find dead ones.

The experiment with the mice partly protected by a "jungle" of sticks extending around the outside border of each testing compartment was then repeated, with the long-eared owl as the predator (Table V). This bird was more timid than was the barn owl and did not secure as many mice per trial period of fifteen minutes. However, he took more of the conspicuous than of the concealingly colored mice over a wide range of light intensities extending from 0.002,3 to 0.000,003,5 foot candle. At intensities of 0.000,6, of 0.000,1, of 0.000,002, and of 0.000,000,53 foot candle, however, the owl took more of the concealingly colored than of the conspicuous mice, though in each case the number of individuals taken was small. In complete darkness the owl secured one mouse in twenty trials, but it seems likely that this mouse was taken at the instant just after the lights were turned on, for the owl was then discovered on the floor of the room with a newly captured mouse in his talons.

If all the mice taken in this experiment at all the light intensities are added together, omitting only the mouse taken in darkness, then forty-three conspicuously colored mice and twenty-six concealingly colored mice were taken. The selection index against the conspicuously colored mice is 0.246. The chi-square of the deviation from a 1 : 1 ratio is 4.188, which is barely significant, presumably because of the few mice included in the counts. The difference in the numbers of mice taken on the silica sand and on the gully soil, respectively, is not significant. Of the ivory mice, however, forty-six were taken against only twenty-four of the discard-grays. The chi-square of the difference is 6.914, which is highly significant, showing that the owl had a preference for the ivory mice.

In order to measure the effectiveness of selection when there is only a small amount of difference between the colors of two contrasting kinds of prey, another experiment was then performed using buff and *blandus*-gray mice. The barn owl was used as the predator, and the mice were exposed as before for fifteen-minute periods. In this experiment, however, the "jungle" of interlaced sticks was extended to cover the whole floor of each testing compartment, excepting only the area occupied by the metal nest boxes of the mice. The gully soil placed in the one compartment closely matched the color of the buff mice, but the Ann Arbor sand in the other

TABLE V

SELECTION OF IVORY AND DISCARD-GRAY DEER-MICE BY LONG-EARED OWL

Partial "jungle" around outside of each compartment. Four live mice of each of two colors exposed in same compartment for each trial; fifteen-minute periods.

Illumination in Foot Candles (Approximate)	Mice Taken									Conspicuous Mice Taken	Concealingly Colored Mice Taken
	On Silica Sand				On Gully Soil				Average per Trial		
	Ivory		Discard-gray		Ivory		Discard-gray				
	Trials	Taken	Trials	Taken	Trials	Taken	Trials	Taken			
0.002,3	5	......	5	1	7	1	7	1	0.25	2	1
0.001,4	4	1	4	2	5	......	5	......	0.33	2	1
0.000,60	9	2	9	2	8	......	8	1	0.29	2	3
0.000,10	6	3	6	......	6	2	6	......	0.42	2	3
0.000,026	10	4	10	......	10	8	10	......	0.60	8	4
0.000,013	10	2	10	3	10	10	10	......	0.75	13	2
0.000,003,5	10	3	10	5	10	7	10	3	0.90	12	6
0.000,002,0	6	1	6	1	5	......	5	2	0.36	1	3
0.000,000,53	6	1	6	......	6	1	6	2	0.33	1	3
Dark	10	......	10	......	10	......	10	1	0.05	0	1
Totals	76	17	76	14	77	29	77	10	....	43	27
Totals omitting "dark"	66	17	66	14	67	29	67	9	....	43	26

compartment did not so closely match the color of the *blandus*-gray mice. Only one light intensity was used, calculated to be 0.000,10 foot candle. In 176 trials, 107 conspicuous and 65 concealingly colored mice were taken (Table VI). The index of selection against the conspicuous mice is 0.244. The chi-square of the deviation is 10.256, which indicates high significance. There is no significant difference between the number of mice of either color taken in the experiment, nor between the numbers which were taken on the two colors of soil.

An important difference in rate of selection, however, appears in this experiment to be correlated with the color of the soil. On the gully soil fifty-five of the conspicuous *blandus*-gray mice were taken, and only twenty-five of the concealingly colored buff mice. The index of selection here is 0.375, and the chi-square is 11.250, which is highly significant. On the Ann Arbor sand fifty-two of the conspicuous buff mice were taken and forty of the presumably concealingly colored *blandus*-grays. The selection index on this soil was only 0.130, and the chi-square only 1.565, which is not significant. The cause of the difference in rate of selection on these two colors of soil is believed to be the better match in color of the buff mice to the gully soil than that of the *blandus*-gray mice to the Ann Arbor sand. It has been pointed out that the *blandus*-gray mice were variable in shade of color, so that some of them did not well match the color of the Ann Arbor sand.

TABLE VI

SELECTION OF BUFF AND *BLANDUS*-GRAY DEER-MICE BY BARN OWL

A complete "jungle" over the whole floor of each compartment. Four live mice of each of two colors exposed in same compartment for each trial; forty-four trials on each soil type. Illumination calculated to be 0.000,10 foot candle.

Mice Taken				Conspicuous Mice Taken	Concealingly Colored Mice Taken
On Gully Soil		On Ann Arbor Sand			
Buff	*Blandus*-gray	Buff	*Blandus*-gray		
25	55	52	40	107	65

These experiments with owls and deer-mice demonstrate that when live deer-mice are exposed on the bare floor of a room without protection of any kind, owls of two species are able to capture them in complete darkness, presumably by the sense of hearing. In every experiment, however, in which the owl was in considerable part dependent upon the sense of sight for capturing his prey, the greater proportion of animals captured was of a color contrasting with that of the background.

In those experiments where the mice were given the protection of a "jungle" of sticks, simulating the protection given to the mice in nature by the vegetation, the selection index in favor of the concealingly colored mice over those that contrasted in color with their background ranged from

0.24 to 0.29. The deviation of the selection index from zero is highly significant in the two experiments in which the barn owl served as the predator. In the one experiment in which a long-eared owl served as the predator the deviation of the selection index from zero is only barely significant (chi-square = 4.188), probably because of the small number of mice involved.

EXPERIMENTS BY SUMNER ON THE SELECTION OF FISH

The best measurements previously published of the effectiveness of selection under carefully controlled laboratory conditions are those of Sumner (1934, 1935*a*, 1935*b*). Sumner did not estimate the effective rate of selection shown by his experiments, but this may be calculated from his figures. In his experiments on the selection of dark-background-adapted and pale-background-adapted mosquito-fish (*Gambusia patruelis*) by the Galapagos penguin, 366 fish were eaten that contrasted in color with their background and 176 that were more or less similar to the shade of their background (Sumner, 1934). If four fish that were of doubtful color are ignored, the total number of fish taken was 542. The selection index against the conspicuous fish is 0.351, and the chi-square of the deviation is 6.661, which is highly significant.

Similarly, when a night heron was used as the predator, 223 conspicuous and 140 protectively colored fish were eaten or injured (Sumner, 1935*a*, Table 2). The selection index in favor of the protectively colored animals is 0.229, and the chi-square is 18.978.

In those experiments in which a sunfish (*Apomotis cyanellus*) was used as the predator only those tests in which the numbers of the two color types of mosquito-fish were equal and in which they were exposed to the predator without a waiting period will be considered (Sumner, 1935*b*: Table 1, experiments 1–4, 9–12, 14, 18, 19, 23, 28). Under these conditions, 166 conspicuously colored and 79 concealingly colored prey were taken. The selection index against the conspicuous fish was 0.355, and the chi-square of the deviation is 30.894.

In these experiments of Sumner the selection index against the animals that were conspicuous when viewed against their backgrounds ranged from 0.23 to 0.36. This is a very considerable amount of selection against the conspicuous fish as compared to the concealingly colored ones. In every experiment the deviation of the selection index from zero is statistically highly significant.

EXPERIMENTS BY ISELY ON THE SELECTION OF ACRIDIANS

Isely (1938) studied the selection of various grasshoppers and other acridians by domestic and wild birds. The prey were sometimes picketed out alive and sometimes were anesthetized. They were exposed in the various experiments to the attacks of bantam chickens, a domestic turkey, English sparrows, mockingbirds, and other birds. Soils of several colors were prepared on plots about sixteen by sixteen inches square arranged in a checkerboard pattern in an open yard at Waxahachie, Texas. The soil colors employed were white, black, red, and green, the last being produced by a cover of vegetation. The acridians used were ordinarily exposed in matched pairs of species, each member of the pair being recorded as being "protected" or "nonprotected" in coloration on the soil concerned.

Inasmuch as different species of acridians were compared when exposed on the various colors of soil, these experiments by Isely were not as carefully controlled as were those of Sumner and of myself, where the two types of prey offered were always of the same species. Where different prey species are compared it is always possible that some difference in their size, shape, or behavior might influence the selection. Furthermore, the soils used by Isely were of several types, on only one of which the particular prey species concerned was concealingly colored. Nevertheless, these experiments are of great interest and value in that a diversity of predators and of prey species were utilized.

Each of the types of predators employed took a greater number of the nonprotected than of the protectively colored species of acridians (Isely, 1938: Table 5). The total of nonprotected acridian individuals taken in all the experiments was 405 and of protectively colored individuals was 183. The selection index against the nonprotected individuals is 0.378, and the chi-square of the index is 83.816.

DISCUSSION

The experiments above described in which owls served as predators and deer-mice as prey demonstrate that on bare ground the barn owl and the long-eared owl are able to capture living prey without the use of vision, presumably by the sense of hearing. Other experiments not previously reported have demonstrated that the barred owl (*Strix varia*) also is able to capture live deer-mice in complete darkness on the bare floor of the reaction room.

In nature, however, wild mice usually avoid open spaces and keep under vegetation, rocks, and other "cover." Although the sense of hearing must be of great value to owls in locating the general position of their prey, it is very doubtful if in nature the sense of hearing alone would suffice for making the actual captures. The well-developed eyes that owls possess and their demonstrated ability to see in very weak light indicate that sight is an important factor in their ecology. It seems certain, therefore, that to secure their prey under natural conditions owls must usually depend heavily on sight.

Birds and teleost fishes both have color vision (Walls, 1942), but it cannot be demonstrated that the predators employed in the experiments by Sumner and myself actually made use of contrasts in hue in capturing their prey. Many of the experiments with the owls were performed under illuminations so weak that the birds could not be expected to discriminate colors. Even in those experiments where there was a difference in hue as well as in shade between the two types of prey offered the owl, it seems probable that the choice must have been based on the difference in shade rather than on the difference in hue. It is noteworthy, nevertheless, that the owls tended to select those mice that to human eyes were conspicuous in hue as well as in shade when viewed against their background of soil.

The range of nocturnal light intensities over which the owls select deer-mice that are conspicuous in color is very extensive. In one experiment with the barn owl (Table IV) selection was indicated to be operative over the whole range of light intensities from just below an illumination at which the mice would be inactive due to the light being too bright, down to the lowest light intensity at which the owl probably could see his prey. The evidence from the long-eared owl is less conclusive (Table V), but indicates that selection for the more conspicuous mice occurred over a considerable range of illuminations.

Within some certain range of light intensities it is possible that the selection of deer-mice by owls may be more intense than under either weaker or stronger illuminations. The previous study of the ability of owls to find dead prey in weak illuminations showed that over a long range of decreasing illuminations these animals have increasing difficulty in finding their food (Dice, 1945). It might be assumed, therefore, that selection should be most discriminating at those light intensities where the predator is just barely able to see his prey. The data available, however, are not sufficient to prove such an effect.

Whether or not selection may be more intense at some intensities of illumination than at others, it is indicated by these experiments that natural selection should be operative under almost any illumination to which deer-mice would likely be exposed in nature.

The difference in pelage color between the ivory and the discard-gray mice used in certain of the experiments (Tables III–V) seems to our eyes to be much greater than that between the buff and the *blandus*-gray mice used in the last described experiment (Table VI). Nevertheless, the selection indexes did not differ very greatly in these several experiments. This may in part be due to the fact that in the experiments with the ivory and discard-gray mice the middle of the testing compartment was bare and was not covered by the mouse "jungle." Some mice were certainly taken in the open area in the middle of the compartment and if these animals were taken without the use of sight, this may have tended to decrease the apparent effectiveness of selection against the conspicuous ivory mice. Furthermore, the amount of data obtained, even with these laborious experiments, is inadequate for statistical comparison.

The difference in pelage color between the buff and the *blandus*-gray mice used in the one experiment (Table VI) is no greater than the differences that in nature distinguish certain subspecies and local races of *Peromyscus*. In areas of intergradation between subspecies and in many other situations, the populations of these deer-mice may be composed of various proportions of two or more color varieties. In fact, buff and gray color phases are conspicuous in the wild populations of the deer-mice of the subspecies *blandus*. It has already been demonstrated that the buff and gray color phases of *blandus* are due to a single gene difference, buff being dominant to gray (Dice, 1933). Furthermore, in the native region of *blandus* in southern New Mexico various colors of soils occur, some of which roughly resemble the gully soil and Ann Arbor sand, respectively, used in this experiment. In many habitats in nature, therefore, the conditions for the incidence of selection closely parallel those provided in the laboratory experiments here reported.

In every population of *Peromyscus* much of the variability that exists has a basis in heredity (Sumner, 1932; Dice, 1940). The variability within populations in pelage color and in other inherited characters provides natural selection with an abundance of materials on which to operate. It is impossible to escape the conclusion that natural selection by predators must be an important factor in the evolution of pelage color in these deer-mice in any region where they are subject to predation by owls or by other predators with similar visual acuity.

The selection indexes in those experiments in which the barn owl and long-eared owl were forced to depend largely on sight for the capture of their deer-mouse prey ranged from 0.24 to 0.29. In the experiments by Sumner, in which mosquito fish were used as prey and the Galapagos penguin, the night heron, and the sunfish were predators, the selection indexes were, respectively, 0.35, 0.23, and 0.36. Isely's experiments on the selection of acridians by birds gave a selection index of 0.38. The similarity in the magnitudes of these indexes, irrespective of the kind of predator or the type of prey, is surprising. The deviations of the indexes from the zero value expected of a 1:1 ratio in the absence of selection is in every case statistically significant and in most of the experiments the chi-square values of the deviations are high.

The most striking feature of all the laboratory experiments here described is the very great advantage the concealingly colored individuals of the prey species have over the conspicuous individuals in escaping capture by predators. In every experiment in which the predator was evidently using sight to capture his prey, the concealingly colored individuals enjoyed more than a 20 per cent advantage over the conspicuous animals in escaping capture. In one of the experiments reported by Sumner the advantage of the concealingly colored individuals amounted to as much as 36 per cent, and in the experiments by Isely the advantage was 38 per cent.

Such a high rate of selection, should it be applied to a natural population, would undoubtedly result in a very rapid change in the frequencies of the genes producing the character under selection. If natural selection operates in nature with anything like the effectiveness indicated by these experiments and if the trend of selection is generally in the same direction, evolution should proceed very rapidly whenever and wherever a variable form is exposed to its action.

SUMMARY

Experiments are described in which it is attempted to measure the rate of selection under controlled laboratory conditions when the barn owl (*Tyto alba pratincola*) and the long-eared owl (*Asio wilsonianus*) serve as predators and the deer-mouse (*Peromyscus maniculatus*) as prey. Equal numbers of two different color varieties of the mice were exposed to the owls for a series of fifteen-minute periods. In most of the experiments a ''jungle'' of interlaced thin sticks gave partial cover to the mice. The mice were exposed alternately on two colors of soil, one type of which matched the color of one variety of mouse and the other soil that of the other kind of mouse.

On the floor of a room free of obstructions the owls are able to capture live deer-mice in complete darkness, presumably by the sense of hearing. Under the partial protection of a ''jungle'' of sticks, however, the owls seldom were able to catch mice except under illuminations at which it had earlier been demonstrated that they can see.

A selection index is proposed to measure the effectiveness of selection. In a situation where two types of prey, A and B, are available in equal numbers, if the numbers taken are respectively, a and b, then the selection index of A is $a-b/a+b$. The selection index may vary between $+1.0$ and -1.0, being zero in the absence of selection. The significance of any given selection index may be tested by chi-square.

In those experiments in which the owls were forced to depend very largely on sight for capturing the deer-mice the selection indexes in favor of the concealingly colored mice ranged from 0.24 to 0.29. Selection occurred over a wide range of illuminations, extending from about the lower limit at which owls are able to see their prey to an intensity of light above which these nocturnal mice usually become inactive. In an experiment in which buff and gray mice with pelage colors similar to those that distinguish races in nature were used as prey, a selection index of 0.24 was obtained in favor of the mice that were inconspicuous when viewed against their backgrounds.

The experiments performed some years ago by F. B. Sumner on the selection by several kinds of predators of light-adapted and dark-adapted mosquito fish give selection indexes ranging from 0.23 to 0.36. The experiments by Isely on the selection of acridians by various kinds of birds show a combined selection index of 0.38.

From the very high possible rates of selection indicated by these experiments, it is concluded that natural selection can theoretically produce very rapid evolution whenever a genetically variable population is exposed to its action.

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CONTRIBUTIONS FROM THE LABORATORY OF VERTEBRATE BIOLOGY

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AN ANALYSIS OF CERTAIN GENETIC VARIATIONS IN PELAGE COLOR OF THE CHIHUAHUA DEER-MOUSE (*PEROMYSCUS MANICULATUS BLANDUS*)

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INTRODUCTION

GENETIC analysis of the differences between wild-type individuals comprising local populations, races, and species of animals is a necessary step toward understanding the complex process of evolution of animal kinds. Laboratory studies of mutant characters must be extended to include the attributes that differentiate between free individuals and taxonomic groups if genetics is to make its most effective contribution toward solving the problems of evolution.

Wild genotypes are more difficult to analyze genetically than are the usual laboratory-bred strains of animals. The wild genotype is a complex system of major and minor genes that has been molded into attunement with its environment by the forces of mutation pressure, gene dispersal, and local selection pressures. The effects of major genes in wild populations consequently may be obscured by the accumulated effects of other minor, modifying genes. The obvious method of attack is to bring the animals into the laboratory and there to establish homogeneous strains that exhibit the characters of the wild populations. This is the approach used in the present undertaking.

This report deals with genetic analysis of certain wild-type variations in pelage color of the deer-mouse (*Peromyscus maniculatus blandus*). This particular animal was chosen because the variable deer-mouse populations of the southwestern deserts provide the best material for such a study. Even small local populations in the desert contain mice of sharply different hues and shades of color. The magnitude and relative discontinuity of these differences suggest the possibility of single gene differences in major color characters.

The stock of deer-mice treated here came from the Tularosa Basin of southern New Mexico. Buff and gray color phases were recognizable in the original sample of the wild population. These buff and gray mice were used to establish laboratory populations of the corresponding color types. The number of color strains was later increased as other color differences were recognized. The several stocks were then crossed to determine the mode of inheritance of the different color characters.

The buff-gray dimorphism of deer-mice has received some attention in the past. Osgood (1909) observed buff and gray forms of deer-mice in four different geographic races. Collins (1923) showed that the differences between buff and gray are genetic. Dice (1933) crossed buff and gray deer-mice and showed that buff and gray are alternative characters of which buff is dominant over gray. The present study further tests the mode of inheritance of buff and gray coat color and also deals with other variations in pelage color of these deer-mice.

ACKNOWLEDGMENTS

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METHODS

The parent stock of deer-mice was trapped alive about three miles north of Tularosa, Otero County, New Mexico, from October to December, 1938. The field-caught sample shipped alive to the Laboratory of Vertebrate Biology comprised 108 deer-mice. These mice and their offspring were kept under standard laboratory conditions and were fed a standard ration. The offspring of the original, field-caught mice were used to establish the color strains. Inbreeding was held to a minimum during selection within the color stocks, for even a few generations of close inbreeding produces a high degree of infertility in *Peromyscus*. As a rule, any intra-sibship matings were followed by inter-sibship matings between similar phenotypes as approximated by visual inspection.

The offspring of crosses between the color strains were classified as to color type while the mice were alive. Then the mice were prepared as flat skins at an age of approximately five months. The mice were classified again into color types after the skins were prepared. No important difference was found in the color identifications of the living mice and the prepared skins.

Gross color analyses of the several strains and the offspring of the crosses have been made with a Photovolt photoelectric reflection meter, model 610. This instrument consists of a galvanometer and a search unit. The light source is a six to eight volt, fifteen candle-power headlight bulb. The light reflected from the chosen area of the mouse skin stimulates a photoelectric cell. The electric current produced by this cell, when stimulated by reflected light, is read on a sensitive galvanometer. The search unit is placed on a block of white magnesium carbonate, and the galvanometer needle set at 100 before each reading on a skin. The zero point is standardized by projecting the light from the search unit into a tube of dull black paper. The galvanometer reading, then, is the percentage of light reflected from the skin as compared to the amount reflected from the magnesium block. The aperture of the search unit is masked to pick up the light reflected from an area of 9.3 by 7.5 mm. All reasonable precautions have been taken to eliminate stray light and to prevent mechanical error.

Color readings have been made routinely on the dorsal stripe of the flat skins in approximately the middle of the back. For some comparisons readings also have been made on the colored area of the side. A red filter was used alone for readings on the dorsal stripe when the comparisons between stocks principally involved differences in shade and saturation. When a measure of the qualitative differences between stocks was desired, red, green, and blue filters were used for both mid-dorsal and side readings. All color readings have been made by the author or by an assistant, William Jalosky. Repetition of random samples of the other's work by each of the operators indicates the reliability of this method of color measurement.

The reflection-meter values are not directly comparable with color measurements made with the Ives tint photometer originally applied to mammalian colorimetric work by Sumner (1927) and subsequently used in many studies of mammalian pelage color (Dice, 1932). The greater objectivity and ease of operation of the reflection meter make its use highly preferable to use of the tint photometer.

No attempt has been made to analyze the minute details of the hair structure and pigmentation of the color strains. Sample hairs from the several stocks have been examined, however, to determine their major differences in pigmentation. Hairs have been taken for examination from the hip region, the dorsal stripe in the lumbar region, and from the vicinity of the root of the tail of sample dried skins. The hairs were soaked overnight in xylene to eliminate air spaces, then they were mounted in clarite for microscopic examination. The major details of the hair pigmentation agree with those described by Collins (1923), with one exception mentioned below.

Only two types of pigments are present in the deer-mouse hairs, but each of these varies greatly in its intensity. The yellow-orange (yellow of Collins) pigment ranges from pale lemon yellow to intense yellow-orange. The sepia pigment varies from pale brown in some stocks to black in others. My observations agree in part with those of Collins (1923) that the intensity of the pigment appears to be proportionate to the size of the granules and the density of the masses in which they occur. The granules of pale sepia are possibly also qualitatively paler than are the dark sepia granules. I do not agree with Collins (1923) that the yellow-orange occurs in both granular and diffuse form, as this pigment appears granular in our material. Collins' observation of diffuse pigment possibly results from his use of glycerin as a mounting medium. It has been recently observed by Russell (1946) that glycerin sometimes reacts with yellow pigment in the house mouse (*Mus musculus*). This leads to a gradual breakdown of the yellow granules, leaving the cells filled with diffuse yellow coloration.

The chi-square test has been used to determine the significance of deviations from the expected ratios in F_2 and backcross generations. Comparisons between means have been made by the method of Dice and Leraas (1936). Differences between means which are greater than 2.7 times the standard error of their difference are considered significant.

COLOR STRAINS

Five selection lines were established by using the offspring of the field-caught deer-mice. These lines represent four major phenotypes. The fifth line is accounted for by selection in opposite directions (that is, toward paleness and darkness of pelage) within the limits of one of the major color types. All lines eventually bred true to type except for minor variations in saturation and brilliance of color. The color strains are described as follows:

Black-tipped buff. The dark buff mice of this strain do not differ in appearance from dark buff mice in the original sample of the wild population. In gross appearance, the dorsal pelage is a dark, reddish buff liberally sprinkled with black or dark brown guard hairs. The tail stripe is sharply defined and intensely black. Selection was attempted toward an increase in the saturation of the buff color, but the saturation of some of the field-caught mice never was exceeded. The juvenile pelage usually is distinctly buffy, and many of the young can be identified as soon as the hair appears.

In shade of pelage color, as shown by reflection-meter readings for mid-dorsal red, the black-tipped buff strain is darker than any except one strain of black-tipped gray (Table I). The readings for reflected red on the dorsal stripe among ninety-four specimens prepared at random from the maintenance stock average 14.86 ± 0.25 reflection-meter units. This is slightly, but significantly, darker than the mean of eighty-two field-caught buffs, for the average of the field-caught mice is 16.12 ± 0.27 units, and the difference in the means is 1.26 ± 0.36 units.

TABLE I

SUMMARY OF PELAGE CHARACTERS OF COLOR STRAINS

Strain	Gross Appearance of Pelage	Shade of Pelage Reflection-Meter Readings (Reflected Red)	Characters of Hairs		
			Body (Agouti) Hairs		Guard (Nonagouti) Hairs
			Proximal Band	Subterminal Band	
Black-tipped buff	Dark buff. Tail stripe black, sharply defined	14.86 ± 0.25	Large, black clumps sepia pigment	Intense yellow-orange pigment; scattered granules of sepia	Dark brown or black cortical pigment at tip; black masses of sepia throughout
Black-tipped gray (dark)	Dark gray. Tail stripe black, sharply defined	12.85 ± 0.32	Large, black clumps sepia pigment	No discernible yellow-orange pigment; granules or small clumps of sepia	Dark brown or black cortical pigment at tip; black masses of sepia throughout
Black-tipped gray (pale)	Pale gray. Tail stripe gray to black, not sharply defined	21.60 ± 0.24	Moderately large, black clumps sepia pigment	No discernible yellow-orange pigment; granules or small clumps of sepia	Dark brown or black cortical pigment at tip; black masses of sepia throughout
Brown-tipped buff	Yellow. Tail stripe pale brown	34.15 ± 0.23	Small, brown, granular clumps sepia pigment	Pale yellow-orange pigment; scattered granules of sepia	Pale brown cortical pigment at tip; small, brown clumps of sepia throughout
Brown-tipped gray	Pale brown. Tail stripe pale brown	30.57 ± 0.33	Small, brown, granular clumps sepia pigment	No discernible yellow-orange pigment; scattered granules of sepia	Pale brown cortical pigment at tip; small, brown clumps of sepia throughout

The hair pigments of the black-tipped buff mice are intensely dark, as would be expected from the gross appearance of the pelage. The proximal band of the body, or agouti, hairs contains large, black clumps of sepia pigment. The subterminal band has intense yellow-orange pigment and scattered granules of sepia. The sepia pigment in the cortex of the terminal band varies from dark brown to black in appearance. The guard, nonagouti, hairs have black masses of sepia throughout their length, and the cortical pigment at the tip is dark brown or black.

Black-tipped gray (dark selection line). The dark gray mice of this strain do not differ in appearance from gray field-caught mice except for their greater darkness. The dark gray pelage has a somewhat buffy-brown tone, and close observation reveals numerous black or dark brown guard hairs. The tail stripe is intensely black and sharply defined, as in the black-tipped buffs. Selection in this line was toward darkening the shade of pelage color and eliminating the buffy-brown tone.

The mice of this strain are darker than those of any other line. The readings for reflected red on the mid-dorsal stripe among forty-one individuals taken at random from the maintenance stock average 12.85 ± 0.32 reflection-meter units (Table I). These mice average slightly, but significantly, darker than the black-tipped buff mice, for the difference between their means is 2.01 ± 0.40 units. This gray strain averages darker than the field-caught group of seventeen grays. The field-caught grays average 16.47 ± 0.54 units, and the difference between the two means is 3.61 ± 0.63 units.

These mice differ principally from black-tipped buffs in lacking discernible yellow-orange in the subterminal band of the body hairs. Instead of yellow-orange pigment, the subterminal band in the gray mice contains scattered granules or small masses of sepia. The buffy-brown tone of the black-tipped grays appears to result from the small masses and granules of sepia in the subterminal band. Small quantities or loose clumps of sepia appear brown under the microscope regardless of where they occur. The pigmentation of the proximal and terminal bands of the agouti hairs and the pigmentation of the guard hairs do not differ from that of the corresponding bands in black-tipped buff mice.

Black-tipped gray (pale selection line). In these pale gray mice, as in the preceding stocks, the guard hairs of the dorsal surface appear black or dark brown. The tail stripe is less intensely black and less sharply defined than in the black-tipped buffs and black-tipped dark grays. This pale stock was selected toward increased brilliance of gray. Selection was effected to the point at which the shade of pelage color of the darkest mice of this strain barely overlapped the range of the palest mice of the dark gray strain.

The average shade of pelage color in this strain is shown by reflection-meter readings for mid-dorsal red on eighty mice from the maintenance stock (Table I). The mean is 21.60 ± 0.24 reflection-meter units. These mice are considerably paler than the field-caught grays from which the line originated, for the difference in the means of the two groups is 5.13 ± 0.59 units.

This strain, like the dark gray line, differs from buff mice in lacking any discernible yellow-orange pigment in the subterminal band of the body hairs. The pale gray appear to differ from the dark gray mice in a general, although fairly slight, reduction of the size and density of the masses of sepia pigment. The subterminal band on the agouti hairs of the pale gray mice contains fewer granules of sepia and fewer and smaller clumps of the same pigment.

Brown-tipped buff. These mice are pale buff or yellow-orange in gross appearance. The tips of the guard hairs are pale brown, and these pale tips contrast only slightly with the ground color contributed by the body hairs. The result is a pelage that is much more uniform in color than is the pelage of the black-tipped buffs and grays. The tail stripe is pale brown and indistinctly defined. In juvenile pelage these mice are pale buff, and the young can be distinguished from the young of black-tipped mice as soon as the hair appears.

The original brown-tipped buff mice appeared as segregants in routine crosses between black-tipped buffs. The ancestry of these first brown-tipped buffs traces back to four field-caught, black-tipped buff mice, but the pedigrees do not show how many of the field-caught mice were heterozygous for brown-tipped. Little selection was made in the brown-tipped buff strain. The stock was maintained at a moderate intensity of yellow-orange, although occasional variants both above and below this intensity would have permitted some selection in either direction.

In shade of pelage color the brown-tipped buff is the palest of all the strains. Reflection-meter readings for reflected red on the mid-dorsal surface of 104 mice from the maintenance stock average 34.15 ± 0.23 units. The darkest of these is much paler than the palest of the field-caught mice. The palest field-caught mouse, a black-tipped buff, gives a reflection-meter reading of only twenty-one units; the darkest of the brown-tipped buffs gives a reading of twenty-nine units.

In the details of hair pigmentation, the brown-tipped buff mice differ greatly from the strains described above. Like the black-tipped buffs, the brown-tipped buffs have conspicuous yellow-orange pigment in the subterminal band of the body hairs. This pigment, however, is lemon yellow in contrast to the intense yellow-orange of the black-tipped buffs. The chief difference between the brown-tipped and black-tipped strains is in the density and size of the clumps of sepia pigment in all of the pigmented hairs. In the brown-tipped mice the clumps of sepia are smaller, and the individual granules making up each clump are less compacted than they are in the black-tipped mice. The loosely gathered granules comprising a clump are separately identifiable, and as a result each clump is extremely granular in appearance. The loose arrangement of the sepia granules produces a pale brown color, which contrasts with the black or dark brown of black-tipped mice. This pale brown is found in both the medulla and cortex of the hairs of brown-tipped mice. The pale brown tips of the guard hairs, as seen with the naked eye, result from the paleness of the sepia pigment in the cortex.

Brown-tipped gray. These mice are pale brown in general appearance. The coat of hair is relatively uniform in color, as is the case in brown-tipped buffs. The tail stripe is pale brown and indistinctly defined. The juvenile pelage is pale brown, and these mice, like the brown-tipped buffs, can be identified as soon as the hair grows out.

The original mice of this strain appeared as segregants in two pairs of matings between mice of the black-tipped, pale-gray line. Others were obtained by crossing brown-tipped buffs with black-tipped, pale gray mice and removing the brown-tipped grays in the F_2 generation. The brown-tipped grays trace back to the same field-caught ancestors as the brown-tipped buffs. One brown-tipped gray mouse was trapped in the wild. This was an immature mouse about three weeks of age, which died in the trap and was preserved as a flat skin. The brown-tipped gray line was rigorously selected for paleness.

Some measure of the qualitative color differences between brown-tipped gray and brown-tipped buff is afforded by reflection-meter readings with three color filters, red, green, and blue (Table II). The buffs had significantly higher values for red, both mid-dorsal and side, than did the grays. The gray mice, on the other hand, gave significantly higher readings for green and blue than did the buffs. The greatest difference between the two strains is with reflected blue from the side, where the difference in the means is 5.89 ± 0.33 reflection-meter units. The brown-tipped buff mice, then, are relatively high in reflectance of light rays falling in the red band of the spectrum and low in reflectance of greens and blues. The brown-tipped grays are relatively low in reflectance of red rays and high in reflectance of greens and blues. This is what one would expect from the gross appearance of the pelage of the two strains.

The pigmentation of the hairs in this strain is the same as in brown-tipped buffs with one important exception (Table I). The subterminal band of the agouti hairs, as in the black-tipped grays, lacks discernible yellow-orange pigment. Granules of sepia pigment are scattered through the subterminal band of the body hairs. The sepia pigment of the brown-tipped grays is pale brown, like the sepia pigment of brown-tipped buffs.

Brown-tipped buff and gray mice of my strains have been compared with specimens of recessive "brown agouti" deer-mice described by Huestis and Barto (1934) from a stock of *Peromyscus maniculatus gambelii* originating at Silver Lake, Oregon, and both strains are alike in their pale brown sepia pigments. Crosses made by Elizabeth Barto (unpublished) between my

TABLE II

REFLECTION-METER READINGS ON BROWN-TIPPED BUFF AND GRAY MICE OF SELECTION LINE STOCKS

Means, Standard Errors and Differences

Line	Mid-Dorsal			Side		
	Red	Green	Blue	Red	Green	Blue
Brown-tipped Buff	34.15 ± 0.23	18.55 ± 0.16	11.89 ± 0.14	45.50 ± 0.27	27.50 ± 0.24	18.11 ± 0.19
Brown-tipped Gray	30.57 ± 0.33	19.59 ± 0.20	14.74 ± 0.20	42.01 ± 0.34	30.19 ± 0.28	24.00 ± 0.27
Differences of Gray from Buff	− 3.58 ± 0.40	+ 1.04 ± 0.26	+ 2.85 ± 0.24	− 3.49 ± 0.43	+ 2.69 ± 0.37	+ 5.89 ± 0.33

"brown-tipped gray" from Tularosa and "brown agouti" mice descended from those from Silver Lake produced an F_1 generation of twenty-nine brown-tipped mice and an F_2 generation of thirty-eight brown-tipped mice. The characters "brown-tipped" and "brown agouti," therefore, appear to be produced by the same gene or by alleles so similar that no difference can be recognized. The original "brown agouti" was, from its description, a brown-tipped buff of my nomenclature.

MODE OF INHERITANCE OF MAJOR COLOR CHARACTERS

Cross matings between the stocks were made in various combinations to determine the mode of inheritance of the distinctive color characters of these strains. The major differences between buff and gray deer-mice already have been shown to be due to the action of a single pair of alleles (Dice, 1933). These genes have been designated agouti buff (A^b) and gray (A) by Clark (1938). As it is unlikely that these genes are homologous with the agouti genes of the house mouse, it seems preferable to designate the deer-mouse genes by the symbols (G) and (g), respectively. My recessive strains, brown-tipped buff and brown-tipped gray, appeared to be parallel, pale modifications of buff and gray. It is possible, but by no means certain, that black-tipped and brown-tipped are homologous of black and brown of the house mouse. On the assumption that they are homologous they are here given the symbols (B) for black-tipped and (b) for brown-tipped. The cross matings were designed, therefore, to test the hypothesis that the major color differences in the Tularosa stock are produced by two pairs of alleles. The results of the crosses between my color strains are summarized in Table III.

Brown-tipped gray, when crossed with brown-tipped buff, produced brown-tipped buff offspring with the exception of one brown-tipped gray, which indicates that one buff parent was heterozygous for buff-gray. Matings between the buff F_1 mice resulted in an F_2 generation of sixty-four brown-tipped buff and twenty-eight brown-tipped gray mice. The frequencies of the two phenotypes showed no significant deviation from the expected frequencies in a cross involving a single pair of alleles (Table III). Some of the F_1 progeny were backcrossed to brown-tipped gray. The offspring of this backcross comprised sixty brown-tipped buffs and forty-nine brown-tipped grays. The frequencies of the two classes showed no significant deviation from the expected 1:1 ratio for a simple backcross. This is evidence that brown-tipped buff and brown-tipped gray are homozygous for the brown-tipped gene and that buff is a single unit dominant over gray.

When brown-tipped gray was crossed with black-tipped dark gray, an all black-tipped gray F_1 generation was produced. The F_1 mice were somewhat paler than their black-tipped gray parents, but there was no doubt about their being black-tipped rather than brown-tipped. This effect on shade of color in all crosses between black-tipped and brown-tipped mice will

TABLE III

Segregation in Crosses Between Black-Tipped (*B*) and Brown-Tipped (*b*) and Buff (*G*) and Gray (*g*) Strains of Deer-Mice (*Peromyscus maniculatus blandus*)

Crosses	F₁			F₂			
	Expected No.	Phenotypes	Observed No.	Expected No.	Classes of Phenotypes	Observed No.	χ^2
Brown-tipped gray				69.00	*b G*	64	
×	16	*b G*	15				1.149
Brown-tipped buff		*b g*	1*	23.00	*b g*	28	
Brown-tipped gray				81.00	*B g*	80	
×							0.049
Black-tipped gray (dark)	46	*B g*	46	27.00	*b g*	28	
Brown-tipped buff	61	*B G*	60	48.00	*B G*	49	
×							0.083
Black-tipped buff		*B g*	1*	16.00	*b G*	15	
Brown-tipped buff				105.00	*B G* and *B g*	115	
×							3.809
Black-tipped buff (heterozygous for gray included)				35.00	*b G* and *b g*	25	
Brown-tipped gray				114.75	*B G*	110	Interaction
×				38.25	*B g*	44	
Black-tipped buff	40	*B G*	40	38.25	*b G*	38	1.281
				12.75	*b g*	12	
Brown-tipped buff	20	*B G*	19	54.00	*B G*	50	Interaction
×				18.00	*B g*	20	
Black-tipped gray (dark)		*B g*	1*	18.00	*b G*	21	2.722
				6.00	*b g*	5	
Black-tipped buff				9.25	*B G*	11	
×	22	*B G*	23	18.50	*B G*†	17	
				9.25	*B g*	9	
Black-tipped gray (dark)		*B g*	9*				
BACKCROSS							
F₁ (brown-tipped gray × brown-tipped buff)				54.50	*b G*	60	
×						49	1.111
brown-tipped gray				54.50	*b g*		

* Due to some heterozygosis in parental stocks; not used to produce F_2, nor backcrosses.

† Difficult to classify; possibly heterozygotes.

be discussed later. The F_2 generation contained eighty black-tipped grays and twenty-eight brown-tipped grays. This ratio does not depart significantly from the expected 3:1 ratio in a cross involving a single pair of Mendelian alleles (Table III). It seems evident that black-tipped and brown-tipped gray are homozygous for recessive gray and that black-tipped is dominant over brown-tipped.

The crossing of brown-tipped buff with black-tipped buff produced black-tipped buff offspring with the exception of one black-tipped gray. Since the same level of homozygosity for buff had not been obtained in these strains as in those used in other crosses, the F_2 generation contained some black-tipped and brown-tipped grays. In some sibships only buffs appeared. The frequencies of forty-nine black-tipped and fifteen brown-tipped buffs in this part of the F_2 generation were close to the expected 3:1 ratio for a simple Mendelian cross (Table III). In the total F_2 group the frequencies of 115 black-tipped (including both buffs and grays) and twenty-five brown-tipped (buffs and grays) deviate somewhat from the expected 3:1 ratio. However, this deviation is not considered significant, as the chi-square value is only 3.809 with one degree of freedom. The results of this cross show that black-tipped buff and brown-tipped buff have the gene for buff in common and that black-tipped is dominant over brown-tipped.

TABLE IV

TEST FOR LINKAGE BETWEEN BUFF—GRAY ($G\,g$) AND BLACK-TIPPED—BROWN-TIPPED ($B\,b$)

Test-cross

($B\,G/b\,g \times b\,g/b\,g$)

Class	Observed	Expected
Black-tipped, buff	66	59.25
Black-tipped, gray	63	59.25
Brown-tipped, buff	54	59.25
Brown-tipped, gray	54	59.25
Total	237	237.00

Analysis of Chi-square

Source of Variation	Degrees of Freedom	Chi-square
Black-tipped: brown-tipped ratio	1	1.861
Buff: gray ratio	1	0.038
Linkage	1	0.038
Total	3	1.937

Conclusive evidence that the major characters of the color strains result from the action of two pairs of alleles is found in the cross between brown-tipped buff and black-tipped gray. All F_1 progeny were black-tipped buff except for one black-tipped gray assumed to be indicative of a heterozygous parent. Black-tipped buff is a recombination class here, as the gene for black-tipped must have come from one parent and the gene for buff from the other. The black-tipped buff F_1 produced an F_2 generation of fifty black-tipped buffs, twenty black-tipped grays, twenty-one brown-tipped buffs, and five brown-tipped grays. The frequencies of these phenotypes in

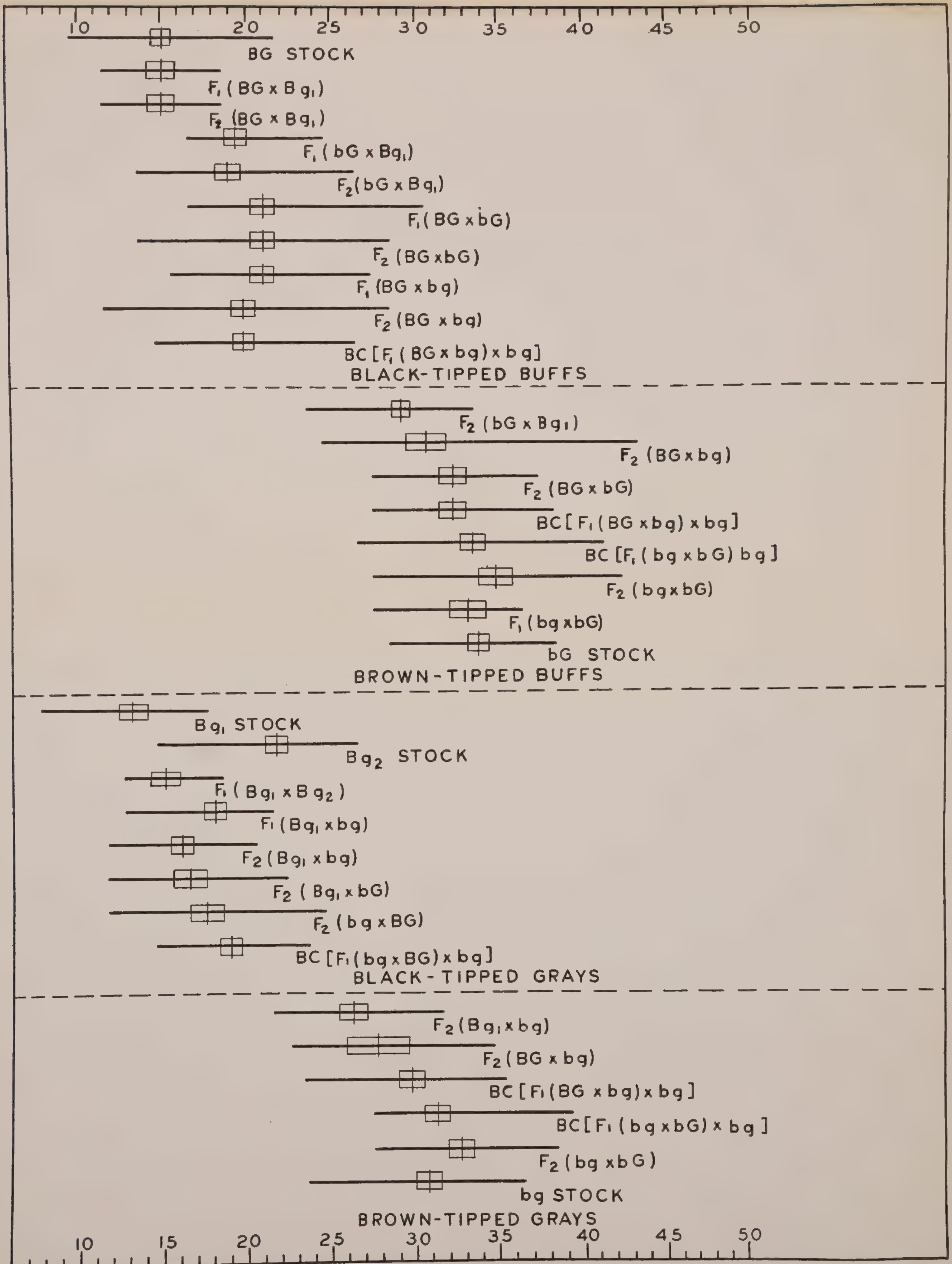

FIG. 1. The mean shade of color of the different classes of segregants, as indicated by reflection-meter readings for mid-dorsal red, in the crosses between *blandus* color strains. The graph is made after the method of Dice and Leraas (1936). The length of each line represents the extremes of that set of measurements. The middle crossbar represents the mean. Two other crossbars are placed two times the standard of the mean above and below the mean, respectively, forming a rectangle. If the rectangles of two comparable lines do not overlap in horizontal position the two means are indicated to differ by a statistically significant amount. Genetic symbols are: black-tipped and brown-tipped (*B b*) and buff and gray (*G g*). A subscript one following the symbol for gray (i.e., g_1) indicates a dark selection line; a subscript two following the symbol for gray (i.e., g_2) indicates a pale selection line.

the F_2 showed no significant deviation from the expected 9:3:3:1 ratio (Table III). Brown-tipped gray and black-tipped buff are recombination classes.

Additional evidence that we are dealing with two pairs of alleles is afforded by the cross between brown-tipped gray and black-tipped buff. This cross produced black-tipped buff offspring. The F_2 generation comprised 110 black-tipped buffs, forty-four black-tipped grays, thirty-eight brown-tipped buffs, and twelve brown-tipped grays. These frequencies showed no significant deviation from the expected 9:3:3:1 ratio (Table III). The brown-tipped buff and black-tipped gray are recombinations.

Some of the F_1 progeny of the cross between brown-tipped gray and black-tipped buff were backcrossed to brown-tipped gray. This backcross affords a test for linkage between the black-tipped, brown-tipped alleles (B b) and the buff, gray (G g) genes. The test is in the coupling relationship. The test-cross generation comprised 237 mice (Table IV). Parental types (black-tipped buff and brown-tipped gray) and recombinations (black-tipped gray and brown-tipped buff) occurred in the progeny in frequencies of 120 and 117, respectively, which do not depart significantly from the 1:1 ratio between parental and recombination types that would be expected in the absence of any linkage between the genes (G) and (B).

The black-tipped buff and black-tipped dark gray strains were crossed and produced both black-tipped buff and black-tipped gray offspring, apparently indicative of considerable heterozygosity in the parental buffs. The buff offspring were duller and grayer than the mice of the parental, buff strain. The buff F_1 mice were mated to produce an F_2 generation of thirty-seven individuals (Table III). In this generation it was difficult to separate all of the progeny into buff and gray classes. Eleven mice, or approximately the expected number of homozygous buffs, were unquestionably buff. Nine, or nearly the expected number of homozygous grays, were unquestionably gray. Seventeen, or approximately the expected number of heterozygotes, were difficult to assign to either class. This apparent suppression of buff dominance in the cross between dark strains will be discussed later.

VARIATIONS WITHIN MAJOR COLOR CLASSES

Only a part of the color variation in the Tularosa deer-mice can be attributed to the effects of the major gene pairs (G g) and (B b). Minor variations in saturation and intensity of color permitted selection within the several color strains. Furthermore, the segregants into major color classes in the crosses showed effects of the crosses that must be attributed to other genes than those determining the major color types. The continuous nature of this variation within color strains leads to the conclusion that a relatively large number of genes with minor, additive effects is involved here. Reflection-meter readings of the shade of color of the segregants in the crosses and of the parental stocks (Fig. 1) provide some measure of the effect of these minor genes.

Before considering the minor variations within the major color types, it is well to recall the nature of selection in the parental strains. The black-tipped buff line was selected toward an increased saturation of buff. The black-tipped dark gray strain was selected toward darkening the shade of the pelage color. These two stocks are presumed to carry a majority of the genes for increased intensity of pigment. Black-tipped pale gray mice were selected toward paleness of pelage. The brown-tipped buffs and grays were selected toward paleness, but selection was more rigorous in the gray than in the buff line. These three stocks that were selected toward paleness are presumed to carry a minority of the intensity genes.

Black-tipped gray was the only major phenotype in which selection was attempted toward both paleness and darkness of pelage. The black-tipped dark grays, derived from the selection toward darkness of pelage, average much darker than the black-tipped pale grays obtained by selection for paleness (Table I). The reflection-meter readings for mid-dorsal red in the black-tipped dark gray strain average 12.85 ± 0.32 units, and the readings for the black-tipped pale gray line average 21.60 ± 0.24 units. The difference between the means is 8.75 ± 0.40 units, which is highly significant. The dark gray strain was crossed with the pale gray line, and an F_1 generation was raised. The progeny were intermediate between the parental stocks in shade of pelage, but they were closer to the parental dark grays than to the pale grays (Fig. 1). It seems evident that the black-tipped dark gray line contains a number of genes for increased darkness of shade, while the black-tipped pale gray line has a reduced number of these minor genes.

When black-tipped mice, presumably carrying a number of minor intensifiers, were crossed with brown-tipped mice, which had been selected against these minor genes, the black-tipped segregants in subsequent F_1, F_2, and backcross generations were always paler in shade than the parental black-tipped mice (Fig. 1). Three types of crosses between black-tipped and brown-tipped resulted in a tipped buff F_1 generation. The cross between brown-tipped buff and black-tipped dark gray had less paling effect on the black-tipped buff F_1 mice than did the other two crosses. The crosses between black-tipped buff and brown-tipped buff and between black-tipped buff and brown-tipped gray produced the palest F_1 black-tipped buffs. In the first of these three crosses most of the minor genes for darkness of shade presumably came from the black-tipped dark gray parent, which had been selected intensively toward darkness of pelage. Therefore, it is to be expected that the cross involving this strain would produce a darker F_1 than would the crosses involving the black-tipped buff strain, which had been selected for saturation of buff rather than total darkness of pelage.

Black-tipped buff segregants in the F_2 generations of the above-mentioned three crosses showed no significant differences in shade of pelage from the corresponding F_1 generations. As would be expected, however, the F_2 generations showed increased variability over the F_1 progeny. A backcross of the progeny of the cross, black-tipped buff × brown-tipped gray, to brown-tipped gray reduced the range of variation in shade but did not change the mean. The black-tipped gray segregants in crosses between black-tipped dark gray and the brown-tipped lines were all paler than the parental black-tipped dark gray strain (Fig. 1). In the cross between black-tipped dark gray and brown-tipped gray, the F_2 black-tipped grays were darker in shade than were the F_1 mice, but they averaged paler than the parental black-tipped dark grays.

Brown-tipped segregants in the F_2 generations of crosses between black-tipped and brown-tipped averaged darker than their respective, parental, brown-tipped strains. The darkest of the brown-tipped gray segregants were in the F_2 generation of the cross between brown-tipped gray and black-tipped gray. The darkest of the brown-tipped buffs likewise segregated out in the F_2 of the cross between brown-tipped buff and black-tipped dark gray. This is additional evidence that the dark-tipped dark gray line carries a larger number of the minor intensifiers of shade than does the black-tipped buff strain.

Summing up this section: (1) Black-tipped strains of deer-mice, which had been selected for darkness of shade of pelage color or for saturation of buff, respectively, showed increased paleness of shade and saturation after crossing to brown-tipped strains that had been selected for relatively pale pelage color, (2) the brown-tipped lines showed increased darkness of shade after crossing to the selected, black-tipped lines, (3) these effects are attributed to the action of several, minor genes with additive effects on the intensity of the sepia and yellow-orange pigment.

DISCUSSION

The major differences in pelage color of the Tularosa deer-mice result from the action of two pairs of alleles. Buff and gray mice differ in the pigmentation of the subterminal band of the agouti, or body, hairs. Buff mice have conspicuous yellow-orange pigment in this band, whereas gray mice lack discernible quantities of yellow-orange. The exact physiologic action of these genes ($G\ g$) is not yet known. Black-tipped and brown-tipped mice differ in the intensity of the sepia pigment in all of the hairs. Black-tipped mice have large black clumps of closely packed sepia granules. Brown-tipped mice have smaller clumps of loosely gathered sepia granules.

Variations within the limits of the major color types appear to result from the effects of an unknown number of genes with minor, additive effects on intensity of pigment. Some of these genes apparently affect only the sepia pigment, and others apparently affect only the yellow-orange. Others possibly affect the intensity of both types of pigment. It is not surprising to find these modifiers of intensity in the deer-mice, for such genes have been long known in such mammals as the house mouse (Grüneberg, 1943) and the guinea pig (Wright, 1942).

The major genes for buff and gray and the minor intensifiers of pigment are principally responsible for the remarkable color variability shown by the species *Peromyscus maniculatus.* The recessive gene, brown-tipped, adds to the potential color variability, but only one homozygote was taken in the wild. The buff and gray genes provide the species with considerable plasticity in regard to hue of pelage. The minor intensifiers provide a wide range of potential variation in shade and saturation of pelage color. The close correlation between the pelage color of deer-mice and the soils inhabited that Dice (1940) found in local populations from widely separated parts of North America requires the plasticity afforded by the minor intensifiers of pigment, in addition to the range of variability permitted by the major genes.

Some of the minor, intensity genes have an interesting effect on the buff phenotype. In wild-caught mice buffs usually cannot be identified as such until the mouse has grown its adult coat of hair. In the black-tipped buff strain, which was selected for saturation of buff color, the buff phenotype can be distinguished from gray as soon as the first coat of hair appears. Some modifying genes, which were apparently lost during selection in the buff strain, presumably delay the deposition of yellow-orange pigment until the growth of the adult pelage. When, as pointed out earlier, these black-tipped buff mice are crossed with black-tipped dark grays, the F_1 buffs are a less bright buff than are the parental buffs. They are duller and grayer in appearance than are mice of the black-tipped buff line, and the buff cannot be distinguished from the gray phenotype until the adult pelage appears. The conclusion seems justified that the gray mice brought into the cross modifiers that had been lost during selection in the buff line.

In the F_2 generation of the cross, black-tipped buff × black-tipped dark gray, the segregation is into three classes with frequencies close to the expected for a case of incomplete dominance (Table III). This departure from the relatively sharp segregation in the other crosses between buff and gray probably results from the action of the minor, modifying genes. The black-tipped dark gray parents presumably brought into this cross a major number of minor sepia intensifiers. The black-tipped buff parents presumably contributed a large number of yellow-orange intensifiers and undoubtedly some sepia modifiers. The yellow-orange pigment seems to be suppressed to some extent in the filial generations of this cross by the effects of the minor, sepia intensifiers. This suppression of the yellow-orange is greatest in the presumed heterozygotes of the F_2 generation, in which a single dosage of the buff gene and presumed recombinations of the minor, sepia intensifiers result in a buffy-gray phenotype. Parallel cases of inability to distinguish between all buff and gray individuals, in dark, wild populations of deer-mice, will be discussed in a later report..

SUMMARY

Color variations in a stock of deer-mice (*Peromyscus maniculatus blandus*) from Tularosa, New Mexico, are accounted for by genes with major and genes with minor effects.

Two pairs of alleles with major effects account for the major color phenotypes in these deer-mice: Buff (*G*), which is characterized by yellow-orange pigment in the subterminal band of the body hairs, dominates gray (*g*), which lacks the yellow-orange pigment. Black-tipped (*B*), in which the sepia pigment appears black or dark brown, dominates brown-tipped (*b*), in which the sepia pigment is pale brown in appearance.

Minor variations within the major color types appear to be due to the actions of several genes with minor additive effects on the intensity of pigment. Some of these genes apparently affect only the sepia pigment, and others apparently affect only the yellow-orange. Some possibly affect both types of pigment. A major accumulation of minor intensifiers of sepia often suppresses the yellow-orange pigment in buff mice.

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ESTIMATED FREQUENCIES OF THE BUFF AND GRAY GENES (*G*, *g*) IN ADJACENT POPULATIONS OF DEER-MICE (*PEROMYSCUS MANICULATUS BLANDUS*) LIVING ON SOILS OF DIFFERENT COLORS

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INTRODUCTION

This report deals with the frequencies of two Mendelian genes which have major effects on pelage color. Gene ratios are estimated for five closely adjacent populations of deer-mice (*Peromyscus maniculatus blandus*) in the Tularosa Basin, New Mexico. The estimated gene frequencies at the five localities are discussed in relation to the color of the soils inhabited by the mice and to the ecological distribution and population dynamics of the species of this area.

Areas of buff and of gray soils occur adjacent to one another in many parts of the Tularosa Basin. Some of the ecological associations on these soil types include considerable populations of deer-mice, and the dynamics and ecological limits of these populations are probably as well known as those of any populations of small mammals. The two genes studied produce a buff and a gray mouse, respectively. The comparative frequencies of these genes for buff and gray pelage on adjacent areas of buff and gray soils should, therefore, afford some measure of the intensity of selection against the mice that are conspicuous on their backgrounds.

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The method for statistical treatment of the data was devised by Charles W. Cotterman, and acknowledgment is gratefully made for his important contribution to the study.

METHODS

Stocks of deer-mice were collected at five localities in the eastern Tularosa Basin from October to December, 1938. The field-caught mice were shipped alive to the Laboratory of Vertebrate Biology at Ann Arbor, Michigan. In the laboratory the buff mice, dominant over gray (Dice, 1933), were mated to gray to determine their genotypes. The offspring of the test cross were classified into color types and then, at an age of approximately five months, prepared as flat skins. The classification was rechecked in the prepared specimens.

LOCALITIES OF ORIGIN OF THE SAMPLES

Samples of the wild populations of deer-mice were obtained at the following localities in the Tularosa Basin:

Tularosa. A total of 108 deer-mice were obtained about three miles north of the town of Tularosa. Deer-mice were concentrated in grassy washes along the right-of-way of the Southern Pacific Railroad and along a few grassy washes and irrigation ditches lateral to the railroad. The soil at this station is dark red.

Alamogordo. This sample of 179 deer-mice was taken about three miles south of the town of Alamogordo. The deer-mice were trapped in an extensive area of mesquite association, where they were numerous. The soil is pinkish gray.

Salinas. Sixty-five deer-mice were trapped along the Southern Pacific right-of-way, seven to twelve miles north of Tularosa. Here, as at Tularosa, the deer-mice were concentrated along the railroad, but the mice were scarcer than they were at the Tularosa station. The soil at this station is pinkish gray.

Lone Butte. A total of fifty-seven deer-mice was obtained in an isolated area of mesquite association covering a few acres about thirteen miles west of the town of Tularosa. The soil is pale pinkish gray.

White Sands. This station is in the interior of the gypsum dunes of the White Sands National Monument. It is in section 19, township 17s, range

7e. The soil is creamy-white gypsum "sand." Deer-mice were scarce here, and only thirteen live mice were secured.

ESTIMATE OF GENE FREQUENCIES

As the basis for estimates of the frequencies of the buff and gray genes in adjacent populations of deer-mice, fairly adequate samples from four wild populations and a small and relatively inadequate sample from a fifth population were secured. The gray, or homozygous recessive, mice were determined by inspection. The dominant buff mice then were mated to gray in order to determine whether or not they were heterozygous for gray. Some dominants had no offspring in the laboratory, and a few that did breed produced less than ten offspring. Some of the dominants were mated *inter se* for another study, and in some instances produced litters containing gray offspring, but they later failed to breed when mated to gray mice. The mice of each stock are grouped into three classes for the estimate of gene frequencies. There are (1) the dominants that produced ten or more offspring in crosses to gray, (2) the dominants that produced less than ten offspring when crossed to gray, and (3) the recessive grays, which were determined by inspection.

In estimating the population gene ratio from such data, an approximation may be introduced without appreciable bias or serious loss of precision. I shall ignore the offspring of all buff mice, each of which did not produce ten or more offspring in test crosses with gray, even though some are known heterozygotes. By classifying all such dominants as "untested," a small amount of information is sacrificed, although no bias will be introduced if the progenies are discounted regardless of their composition. Now, the chance that a heterozygous buff mouse should produce ten or more offspring, all buff, is only 1/1024 or less. By classifying such dominants as homozygous, the estimate of gene frequency should be biased in favor of the dominant gene by a small and negligible amount.

Each original sample of wild-caught mice may therefore be classified in four categories: (1) buff, untested, (2) buff, homozygous, (3) buff, heterozygous, and (4) gray. The observed frequencies in these four classes will be designated a, b, c, and d, respectively, their total being n. If all dominants had been tested ($a = 0$), the frequency of the recessive gene, would be estimated as

$$q = \frac{c + 2d}{2n}, \text{ with standard error, } s = \frac{\sqrt{q(1-q)}}{\sqrt{2n}},$$

whereas if all dominants were untested ($b = 0$, $c = 0$), one could take as the estimate, assuming genetic equilibrium,

$$q = \sqrt{\frac{d}{n}}, \text{ with standard error, } s = \frac{\sqrt{1-q^2}}{\sqrt{4n}}$$

(Cotterman, 194~~8~~7). When dominants are only partly classified as to genotype, as in the present instance, a weighted estimate having an intermediate precision is required.

If k represents the proportion of dominants tested, $(b+c)/(a+b+c)$, the expected frequencies (m) in the four classes are as shown in Table I.

TABLE I

EXPECTED FREQUENCIES OF CLASSES OF GENOTYPES WHEN PART OF THE DOMINANTS ARE UNTESTED

Class	Observed Frequency X	Expected Frequency m
Dominants, untested	a	$(1-q^2)(1-k)n$
Dominants, homozygous	b	$(1-q)^2kn$
Dominants, heterozygous	c	$2q(1-q)kn$
Recessives	d	q^2n
Total	n	n

Applying the method of maximum likelihood (Fisher, 1945) to this table, one obtains the estimate

$$q = \frac{\sqrt{(2b+c)^2 + 8n(c+2d) - (2b+c)}}{4n}, \tag{1}$$

and its standard error is given by

$$s = \sqrt{\frac{q(1-q^2)}{2n(k-kq+2q)}}. \tag{2}$$

When $k = 0$ or $k = 1$, these formulae reduce to those given above for the cases of genes with or without dominance, respectively.

If (1) and (2) are applied to the data on buff and gray at each collecting station the estimated frequency (q) of recessive gray genes is found for each of the five populations (Table III). Using these estimates one may calculate the expected frequencies from Table I and test the goodness of fit of the data to the single-gene hypothesis and the assumption of genetic equilibrium (Table II).

Actually, only the assumption of genetic equilibrium is tested here. It is already known that the major differences between the buff and gray mice represent the effects of a single gene (Dice, 1933; Blair, 1947). Therefore, any significant deviations from the expected frequencies may be taken as indications of a departure from genetic equilibrium. Only one degree of freedom remains for the chi-square test since, although there are four classes, the expectations have been made to agree with the observations in respect q to n, ~~[illegible]~~ and k. Applying this test, one finds no significant deviations from the expected frequencies in the four major samples (Table II). All of the χ^2 values fall below 3.841, which marks the 5 per cent level of probability. In the White Sands sample, which is too small for a reliable test of significance, the observed numbers fall fairly close to the expected. It is evident, then, that at least four of the populations sampled are within sample bounds as regards the distribution of genotypes. The character of the fifth, small sample suggests that the population from which it was drawn is also in equilibrium.

COMPARATIVE GENE FREQUENCIES IN THE TULAROSA BASIN POPULATIONS

As an estimate of the frequencies of the buff and gray genes in deer-mice populations of five localities in the Tularosa Basin (Table III) has been obtained, these estimates can be compared to ascertain whether or not the two genes are equally distributed in the total deer-mouse population of the basin (Table IV).

TABLE II

OBSERVED AND EXPECTED FREQUENCIES OF BUFF AND GRAY GENOTYPES IN SAMPLES OF DEER-MICE FROM FIVE STATIONS IN THE TULAROSA BASIN, NEW MEXICO

Sample and Class	Observed Frequency X	Expected Frequency m	$(X-m)^2/m$ χ^2
Tularosa			
Buff, untested	52	51.88	0.0003
Buff, homozygous	14	14.23	0.0037
Buff, heterozygous	22	21.69	0.0044
Gray	20	20.19	0.0018
Total	108	107.99	0.0102
Alamogordo			
Buff, untested	32	31.06	0.0285
Buff, homozygous	4	6.48	0.9491
Buff, heterozygous	43	39.13	0.3828
Gray	97	99.34	0.0551
Total	176	176.01	1.4155
Salinas			
Buff, untested	37	36.68	0.0028
Buff, homozygous	5	5.57	0.0583
Buff, heterozygous	10	9.30	0.0527
Gray	13	13.45	0.0151
Total	65	65.00	0.1289
Lone Butte			
Buff, untested	7	6.61	0.0230
Buff, homozygous	4	6.10	0.7230
Buff, heterozygous	25	21.28	0.6503
Gray	21	23.01	0.1756
Total	57	57.00	1.5719
White Sands			
Buff, untested	2	1.84	
Buff, homozygous	0	0.50	
Buff, heterozygous	4	3.17	
Gray	7	7.48	
Total	13	12.99	

The Tularosa and Salinas samples do not differ significantly in their estimated frequencies of buff and gray genes, and it is inferred that they were drawn from populations having the same parameters in respect to this frequency. Likewise, there is no significant difference in the buff–gray ratio among the Alamogordo, Lone Butte, and White Sands mice. In all other comparisons between any two of the populations the differences in gene ratio

TABLE III

Estimated Frequencies of the Buff (G) and Gray (g) Genes in Populations of Deer-mice (*Peromyscus maniculatus blandus*) at Five Stations in the Tularosa Basin, New Mexico, in Per Cent with Standard Errors

Station	Soil Color	Estimated Frequencies		Standard Errors
		Buff (G)	Gray (g)	
Tularosa	Dark Red	56.76	43.24	3.85
Alamogordo	Pinkish gray	24.87	75.13	2.37
Salinas	Pinkish gray	54.51	45.49	5.10
Lone Butte	Pale Pinkish gray	36.46	63.54	4.61
White Sands	Creamy white	24.13	75.87	8.59

are significant beyond the 1 per cent level of probability. The Alamogordo, Lone Butte, and White Sands mice all have a significantly higher proportion of gray genes than do those from Tularosa and Salinas. The Alamogordo mice have a somewhat, but not significantly, higher proportion of gray genes than do those of the Lone Butte station.

OTHER COLOR VARIATION

The shade of pelage color in the four major samples of deer-mice has been measured by a photovolt reflection meter using a standard technique (Blair, 1947). The reflection meter values are expressed as the percentages of light reflected from designated areas of the dry mouse skin by comparison with the light reflected from a magnesium block. Three color filters, red, green, and blue, have been used.

The buff mice from Tularosa and Salinas do not differ significantly ($t = 2.6$ or more) in any of the color readings (Table IV). Both the Tularosa and Salinas buffs, however, average significantly lower (i.e., darker in shade of pelage) than do the Alamogordo and Lone Butte buffs in all reflection meter readings except dorsal blue. The Tularosa mice to not differ

TABLE IV

Comparison of Buff (G): Gray (g) Ratios in Five Deer-Mouse Populations of the Tularosa Basin, New Mexico

Comparisons	Gene Ratio Differences in Per Cent	t
Tularosa—Salinas	2.25 ± 6.39	0.35
Tularosa—Alamogordo	31.89 ± 4.52	7.06
Tularosa—Lone Butte	20.30 ± 6.01	3.38
Alamogordo—Lone Butte	11.59 ± 5.19	2.23
Salinas—Lone Butte	18.05 ± 6.88	2.62
Salinas—Alamogordo	29.64 ± 5.62	5.27
White Sands—Tularosa	32.63 ± 9.41	3.47
White Sands—Salinas	30.38 ± 9.99	3.04
White Sands—Alamogordo	0.74 ± 8.91	0.08
White Sands—Lone Butte	12.33 ± 9.75	1.26

significantly from either the Alamogordo or the Lone Butte buffs in reflected mid-dorsal blue. The only significant difference between the Alamogordo and Lone Butte buffs is in the value for reflected green; Lone Butte is higher than Alamogordo.

The gray mice from Lone Butte are slightly but significantly higher in readings for mid-dorsal green than are those from Alamogordo. The other gray mice of the four stocks do not differ significantly in any of the color readings. This apparent uniformity of the grays, however, can be given little weight, for the numbers are small in three of the stocks, and the standard errors consequently are large.

From these data, one may deduce that the Tularosa and Salinas samples are drawn from populations having the same parameters for minor, additive genes affecting the saturation and intensity of pigment in the major phenotypes (Blair, 1947). The Alamogordo and Lone Butte samples come from other populations having approximately common parameters in respect to these minor genes.

DISTRIBUTION OF DEER-MOUSE POPULATIONS IN THE TULAROSA BASIN

Deer-mice are widely, but not evenly, distributed in the Tularosa Basin. The deer-mice are limited largely to the mesquite and grassy wash associations, and they occur only rarely in the creosote-bush and atriplex associations (Blair, 1943*a*). Because of strong edaphic controls these ecological associations are discontinuously distributed in the basin. Local areas of mesquite or grassy wash association are separated by extensive areas of creosote bush and atriplex that undoubtedly act as partial barriers to the dispersal of deer-mice. A limited amount of dispersal of deer-mice probably takes place through these unfavorable associations, but it must be of much less magnitude than the dispersal within the limits of a widely distributed favorable ecological community.

The Tularosa, Salinas, and Alamogordo samples constitute a north-south transect through the eastern edge of the basin, parallel to and about three miles distant from the foothills of the Sacramento Mountains. At Tularosa and Salinas the deer-mice were living in grassy washes along a railroad and on the grassy and weedy banks of a few irrigation ditches lateral to the railroad. Creosote-bush association, which is unfavorable to deer-mice, occupies the alluvial slopes adjacent to the foothills and extends into the basin beyond the railroad and is replaced by atriplex association toward the interior. The grassy-wash association inhabited by the mice occupies the floor and sides of a dry drainage ditch paralleling the railroad, and nowhere is the association more than a few yards wide. Extensive trapping in the creosote-bush association resulted in the capture of only two deer-mice. The creosote-bush association is, therefore, at least a partial barrier to the dispersal of deer-mice, and the population along the railroad probably approaches linearity. This condition, of course, has existed only since the

TABLE V

Minor Color Variations in Four Samples of Deer-mice from the Tularosa Basin, New Mexico
Means and Standard Errors of Reflection-meter Readings

Samples and Phenotypes	No.	Mid-Dorsal			Side		
		Red	Green	Blue	Red	Green	Blue
BUFF							
Tularosa	82	16.12 ± 0.27	9.11 ± 0.17	6.61 ± 0.14	28.61 ± 0.41	16.44 ± 0.30	10.78 ± 0.22
Alamogordo	74	19.16 ± 0.27	9.99 ± 0.18	7.36 ± 0.15	31.23 ± 0.36	18.78 ± 0.28	12.70 ± 0.24
Salinas	42	16.14 ± 0.42	9.02 ± 0.27	6.45 ± 0.18	27.95 ± 0.63	16.29 ± 0.37	10.98 ± 0.33
Lone Butte	33	18.45 ± 0.31	11.03 ± 0.23	7.09 ± 0.17	32.06 ± 0.58	19.12 ± 0.43	12.61 ± 0.36
GRAY							
Tularosa	17	16.47 ± 0.54	10.47 ± 0.35	8.41 ± 0.24	29.29 ± 0.77	19.24 ± 0.58	14.65 ± 0.33
Alamogordo	87	17.72 ± 0.23	10.13 ± 0.15	8.80 ± 0.12	30.20 ± 0.36	19.91 ± 0.26	15.59 ± 0.22
Salinas	10	15.60 ± 0.81	10.50 ± 0.46	8.30 ± 0.40	27.90 ± 1.06	19.00 ± 0.96	14.30 ± 0.47
Lone Butte	20	17.60 ± 0.48	11.20 ± 0.38	8.85 ± 0.33	30.15 ± 0.92	21.45 ± 0.62	16.15 ± 0.54

building of the railroad. Before that time, and before irrigation was begun, any deer-mouse population of this section presumably inhabited the grassy washes draining across the creosote-bush association from the foothills of the mountains.

At Alamogordo the mice live mostly in mesquite association. The sample was obtained near the railroad, but the mice were not restricted to the railroad right-of-way as they were at the other stations. The deer-mice were widely and rather evenly distributed over an area of mesquite association that extends several square miles south of the town of Alamogordo. The Salinas station extends along five miles of the railroad from a point four miles north of the Tularosa station to a point nine miles north of that station. The Alamogordo station is eighteen miles south of the Tularosa station. The grassy washes along the railroad provide a possible route of dispersal of deer-mice connecting all of these stations. The unfavorable creosote-bush association flanking the railroad and occupying most of the territory between the stations probably retards dispersal and largely limits it to a linear type by way of the grassy washes along the railroad.

The Lone Butte and White Sands populations are at least partly isolated from the above-mentioned populations of the eastern basin. The interior of the basin exclusive of the White Sands is largely occupied by atriplex association, which is unfavorable for deer-mice. The Lone Butte mice occupied an area of mesquite association that extends only a few acres. It is separated from the Tularosa population, the nearest of the eastern basin populations, by approximately thirteen miles of atriplex and creosote-bush associations. The sparse population of the White Sands, an area of 270 square miles of gypsum dunes near the middle of the basin, is separated from all of the other populations by atriplex and creosote bush.

DISCUSSION

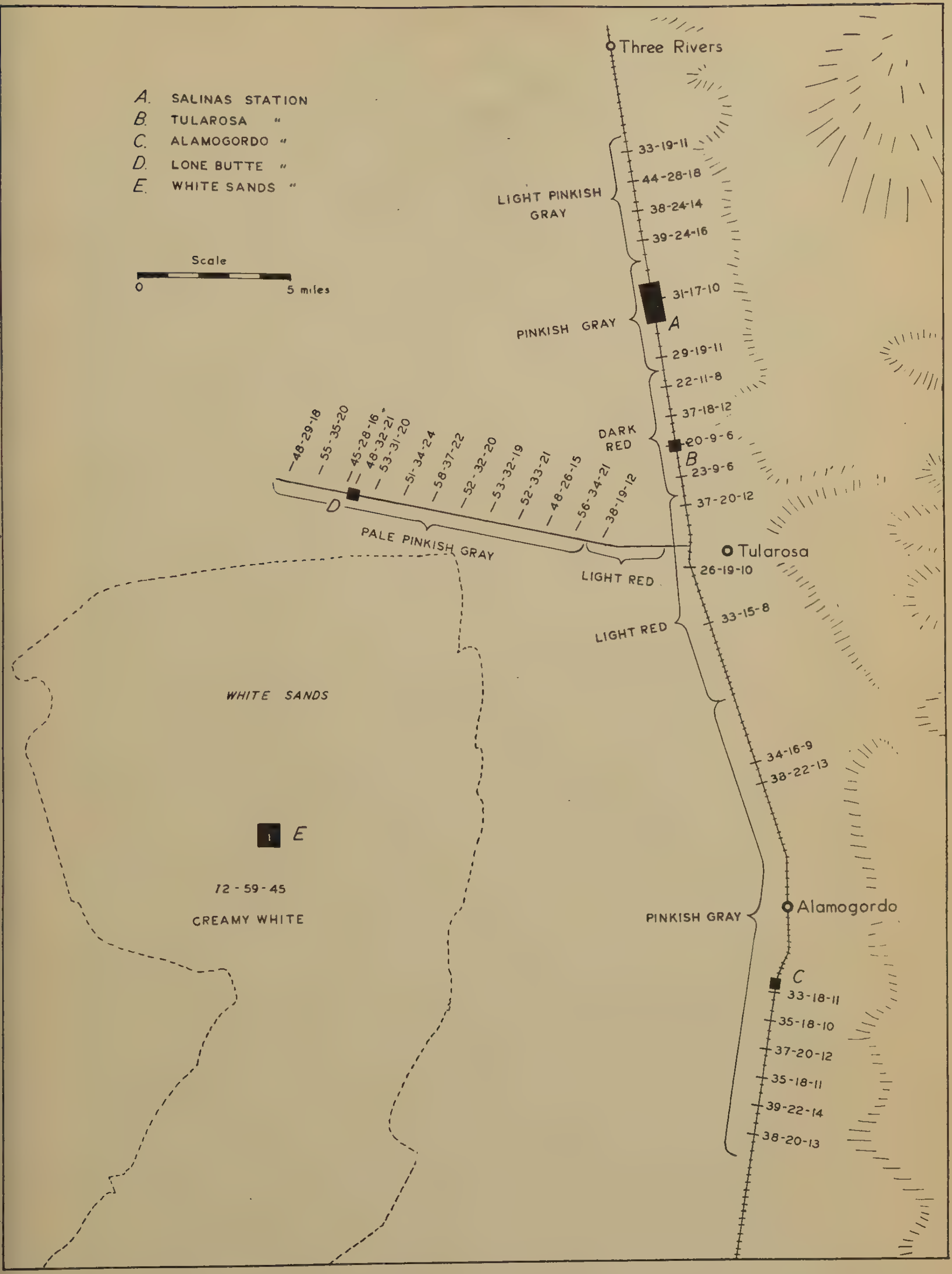
The frequencies of the buff and gray genes in five local populations of deer-mice in the Tularosa Basin have been estimated. I have tested the hypothesis that these genes are in equilibrium in the populations sampled and find that the data support this hypothesis. I have found, further, that the differences between some of the gene ratios exceed the differences that might be expected from sampling variations. Differences in the shade and saturation of the buff phenotype that are attributable to the effects of minor, additive genes have also been found. I have considered the distribution of the deer-mouse population of the Tularosa Basin as it has been determined from ecological studies in the region. An explanation must be sought for the differences and likenesses in buff–gray ratios and for the other genetic color differences within the relatively small area from which the samples were taken.

The genetic differences in the populations sampled might be accounted for in any or all of three ways: (1) isolation by distance and ecological barriers, and consequent random divergence, (2) differential selection pressures at the different localities, and (3) differential mutation rates.

First, there is possibility of random differentiation. Differences in the frequencies of certain gene arrangements in the third chromosome in isolated populations of *Drosophila pseudoobscura* have been attributed to just such random divergence (Dobzhansky and Queal, 1938). The distribution of the deer-mouse population of the Tularosa Basin approximates the "island model" population postulated by Wright (1943) in his mathematical treatment of the effects on gene frequencies of isolation by distance. The local populations are small and at least partly isolated one from the other by ecological barriers. The one probable route of effective dispersal between any of the populations results from recent, man-made changes in the local environment. Therefore, it is possible that random differentiation in partly isolated populations has contributed to the local differences in buff–gray ratios. The difference in gene ratio between Alamogordo and Lone Butte mice (Table IV) possibly results from such random divergence. Mutation rates of the genes under study are unknown, so the possible role of differential mutation rates in the present situation cannot be considered.

Differential selection of the two genes is the third possible cause of the observed differences in gene frequencies. One is dealing here with two genes having major effects on the gross appearance of the mice. The buff gene (G) produces a buff mouse. The gray gene (g), in duplex quantity, produces a gray mouse. It is to be expected that a buff mouse would have a better chance of escaping detection by predators on buff or red soil than would a gray mouse on the same soil. A gray mouse would stand a better chance of survival on gray soil than would a buff mouse. It has been demonstrated recently that under experimental conditions certain predators do capture more gray than buff deer-mice on buff soil and more buff than gray mice on gray soil (Dice, 1947). It should prove profitable, therefore, to examine the colors of the soils on which the Tularosa mice were living for a clue to the reasons for the differences in gene ratios.

Most of the desert soils in this part of the Tularosa Basin are some shade of pinkish gray in appearance (Map 1). The darkest of these pinkish gray soils occur at the edge of the basin, as shown in the transect, south of Alamogordo and from about six miles north of Tularosa north beyond the limits of the transect. Toward the interior of the basin these pinkish gray soils become paler and exceed all other soils of the area in paleness except the creamy white gypsum of the White Sands. In sharp contrast to these pale, predominantly gray soils, there is an area of dark, brick red soil just north of the town of Tularosa. This area of dark red soil is about four miles wide and extends out about five miles from the base of the foothills into the basin. At its northern limit the dark red soil changes abruptly to pinkish gray. At its southern limit the dark red changes abruptly to light red soil that extends south for about seven miles and merges gradually into pinkish gray.



Map 1. Localities at which samples of deer-mouse populations were taken in the Tularosa Basin, New Mexico. Black rectangles indicate collecting stations. Measurements of soil colors (reflection-meter readings) are given for all localities at which soil samples were taken. Values are given for reflected red, green, and blue, respectively.

The Tularosa sample came from about the middle of the area of dark red soil. The relatively high frequency (56.76 per cent) of buff genes and the low frequency (43.24 per cent) of gray genes I believe to be directly related to selection by predators against the nonadaptive gray gene on the dark red soil. In this population the ratio of buff, adaptive to gray, nonadaptive phenotypes, as estimated from the sample, is 81.5 to 18.5 per cent. Actually, the selection has favored not only an adaptive gene but also an adaptive genotype. The buff Tularosa mice are a darker, more saturated buff than are the buff mice from the gray soils, as has been shown by colorimetric methods. These methods, while they do show differences, fail to show the full difference to the human eye between the Tularosa buff mice and most of those from the gray soils. Most of the Tularosa buffs have a dark, reddish buff pelage in contrast to the less saturated and grayer buff of most buff mice from gray soils.

On the dark red soil near Tularosa, I believe that natural selection has favored buff over gray mice and that it has also favored the accumulation of the minor, additive intensifiers of pigment.

The Alamogordo and Lone Butte populations occupy pinkish gray soils. The comparatively high frequencies of gray genes in these populations (75.13 per cent at Alamogordo, and 63.54 per cent at Lone Butte) I attribute to selection by predators against the nonadaptive buff on the pinkish gray soils. As on the Tularosa red soil, selection is favoring an adaptive genotype rather than a single gene. The buff mice of the gray soils are paler than those of the dark red soils. It is possible that once fixation of the buff–gray genes has occurred in these populations of the gray soils, selection has operated principally against the minor intensifiers. It appears that some of the buff mice of the pinkish gray soils, as affected by the minor, modifying genes, are less conspicuous on these soils than are gray mice on the dark red soil.

The high frequency of gray genes (75.87 per cent) in the White Sands population, as estimated from the small sample, possibly indicates the selective advantage of gray over buff on the White Sands. The buff and gray mice are both relatively conspicuous on the white gypsum, and any selective advantage of gray over buff must be of a lesser magnitude than the advantage of one color type over the other on the other soils of the basin. The buff–gray ratio in the White Sands population is to be expected whether or not there is selection for either color type. The White Sands undoubtedly received its original population of deer-mice from near-by populations of the pinkish gray desert soils, and I suspect that the deer-mouse population on the gypsum dunes occasionally approaches zero only to be replenished from populations such as that at Lone Butte. With gene fixation in these populations and with no selection for or against buff or gray on the White Sands, the gene frequencies on the sands would parallel the frequencies in the near-by populations.

The Salinas sample obviously is not consistent with the other samples from the Tularosa Basin. Here, on pinkish gray soil of approximately the same appearance as the Alamogordo soil, there is a buff–gray ratio and an average shade of pelage no different from the gene ratio and the shade of pelage in the population of the dark red Tularosa soil. One must either weaken the case for natural selection as the principal agency in establishing and maintaining the observed gene frequencies in the Tularosa Basin populations or find a reasonable explanation for the situation in this population. The population dynamics of deer-mice in this part of the basin offer such an explanation.

The Tularosa and Salinas samples were taken only four miles apart. There must be relatively free interchange of genes among the deer-mice living in the grassy washes parallel to the railroad. From fragmentary and minimum records it is known that deer-mice frequently move at least one-third of a mile under conditions of moderate population pressure in the mesquite association (Blair, 1943b), and it is likely that they disperse farther than this. Under the conditions of crowding and high population pressure present in the grassy washes, it seems likely that dispersal is over much longer distances.

If natural selection is operating against the buff gene in the Salinas population, then the pressure of immigration from the Tularosa population must be of such magnitude as to obliterate the effects of selection. The buff–gray ratio in the deer-mouse populations along the railroad north of the town of Tularosa consequently would be controlled by selection on the dark red soil. There is other evidence to support this theory. The deer-mouse population at Tularosa was much larger than at Salinas. Only a little more than one-half as many mice were caught at Salinas as at Tularosa (Table II) even though more effort was expended and more territory was covered there than at the latter station. It has been shown experimentally that, given differential population pressures within an ecological association, immigration will proceed mostly from the areas of greater to the areas of lesser pressure (Blair, 1940). There apparently was such a pressure differential between the Salinas and Tularosa populations at the time the samples were taken, and the dispersal pressure from the Tularosa toward the Salinas population probably was considerably in excess of the reverse pressure. The populations were at a peak of abundance when the samples were taken, but this difference in relative population size and a consequent pressure differential would be maintained even under reduced densities of population. It is almost axiomatic that, as population sizes fluctuate, the relative densities of the species in minor subdivisions of a local area maintain their relative proportions. It is quite possible that, with seasonal decreases in population size in the area, the Salinas population becomes very low only to be later replenished by immigration from the relatively larger Tularosa population. The difference in the size of the two deer-mouse populations appears to be due to minor differences in the grassy wash association at the two stations.

The grass cover is thicker at Salinas than at Tularosa, and this condition seemed to favor another, related species. The wood mouse (*Peromyscus leucopus tornillo*) was enormously abundant at Salinas and much less numerous at Tularosa.

The contrasting situations at Salinas and Alamogordo, respectively four miles north and eighteen miles south of the Tularosa station, emphasize the important role of isolation by distance and of population structure in differentiation. Adaptive and even random nonadaptive differentiation may occur under isolation by distance alone (Wright, 1943). Under conditions of partial isolation and selection within the different populations, differentiation will occur faster than under the action of partial isolation or selection alone (Wright, 1938). This situation in nature is illustrative of these processes. The Salinas and Tularosa populations, which are only four miles apart, are estimated to have the same parameters in respect to buff–gray gene frequencies and in respect to minor, modifying, color genes, even though selection pressure is assumed to be exerted in opposite directions in the two populations. I conclude that immigration pressure exceeds selection pressure and that, as discussed above, immigration pressure probably is unequal between the two populations. The Alamogordo population, eighteen miles away, is no more isolated from the Tularosa mice except by distance than is the Salinas population. Thus, while a distance of four miles is not sufficient for differentiation, even under presumably heavy selection pressures, a distance of eighteen miles is sufficient under the conditions in this area, which include differentiation into several vegetation and soil types. The genetic balance between the populations along this transect, as it exists today, probably is influenced by the linear distribution of a part of the populations. Such a distribution of the species favors differentiation more than does areal distribution (Wright, 1943). The frequencies of buff and gray presumably were fixed in the populations long before the railroad was built and under conditions of greater isolation than exist today. The Tularosa and Alamogordo populations do, however, maintain their distinctness, even though a linear route of ready dispersal now exists between their two areas. In the Alamogordo population, then, I conclude that selection pressure balances immigration pressure, whereas in the Salinas population selection pressure is swamped by the pressure of immigration from the more abundant population of the Tularosa red soil.

In summing up this discussion I conclude that the differences in buff–gray gene ratios and the quantitatively determined differences in minor, modifying genes in the Tularosa Basin populations are attributable to: (1) partial isolation of relatively small local populations in favorable ecological associations that are separated by considerable expanses of unfavorable associations, (2) selection by predators against nonadaptive color genotypes, and (3) the combined effects of isolation by distance and ecological isolation. Populations only four miles apart, on differently colored soils, and not isolated, except by distance and relatively linear distribution, show no differentiation in buff–gray gene ratio. Populations eighteen miles apart, on differently colored soils, isolated only by distance, and having a relatively linear distribution do show adaptive differentiation in the frequency of the buff and gray genes.

SUMMARY

Deer-mice (*Peromyscus maniculatus blandus*) were trapped alive at five stations in the Tularosa Basin, New Mexico, and brought into the laboratory for a study of gene frequencies in wild populations. These mice carry Mendelian alleles, buff (G) and gray (g), that have major effects on pelage color, and which presumably have different survival values on differently colored soils.

Genotypes of the dominant buff mice were determined by test crosses in as many of the buff mice as possible. Not all the mice produced offspring, and the gene frequencies were, therefore, estimated by means of a maximum likelihood formula appropriate to data in which only a part of the dominants are classified as to genotype.

A chi-square test of the data revealed no significant departure from genetic equilibrium in any of the populations. Significant differences in buff–gray ratios were present between each of three populations living on pinkish gray soil and each of two other populations living on or near an area of dark red soil.

The differences in gene ratios are attributed to the effects of: (1) partial ecological isolation of the deer-mice into relatively small, local populations, (2) differential selection for the two genes by predators on red and pinkish gray soils, (3) the combined effects of ecological isolation and isolation by distance. Populations only four miles apart showed no differentiation even though selection was presumably operating in opposite directions in the two populations. Differentiation in buff–gray frequencies and in minor color characters was evident in populations fifteen to eighteen miles apart and living on differently colored soils.

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VARIATION IN SHADE OF PELAGE OF LOCAL POPULATIONS OF THE CACTUS-MOUSE (*PEROMYSCUS EREMICUS*) IN THE TULAROSA BASIN AND ADJACENT AREAS OF SOUTHERN NEW MEXICO

BY W. FRANK BLAIR

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INTRODUCTION

THE soils on which cactus-mice (*Peromyscus eremicus*) live in the Tularosa Basin, New Mexico, exhibit extremes in shade of color within a relatively small area. Stocks of cactus-mice were collected in 1938 and 1940 as part of a study of color variation in the mammals of this area. These stocks afford an opportunity to study the relation of shade of soil color to the shade of pelage color of local, slightly isolated populations of cactus-mice.

ACKNOWLEDGMENTS

This study was supported by the Laboratory of Vertebrate Biology, University of Michigan. The 1938 expedition, on which a part of the stocks were obtained, was supported in part by a grant to Lee R. Dice from the Carnegie Institution of Washington. The 1940 expedition, on which other stocks were collected, was supported in part by a grant from the Horace H. Rackham School of Graduate Studies of the University of Michigan. The color readings were made by S. L. Bellanca, D. M. Clarke, C. W. McCollum, W. F. McNally, and myself. The statistical calculations were made by D. M. Clarke, Bruce Kirchenbaum, and myself.

METHODS OF STUDY

The cactus-mice taken in the field were shipped alive to the Laboratory of Vertebrate Biology at the University of Michigan in Ann Arbor. All of the mice were kept under standard conditions and were fed a standard ration. The laboratory-born offspring of these field-caught animals were raised to an age of twenty-two or more weeks, at which time they had attained adult pelage. Then they were killed and prepared as flat skins. The shade of color of the skins was measured by use of the Ives tint photometer, using the methods described by Dice (1932). As the shade of pelage color only was to be measured, tint photometer readings on the skins were made with only the red filter.

COLLECTING STATIONS

Cactus-mice were collected on two lava beds in and near the Tularosa Basin and from three stations on the sandy desert of the same basin (Map 1). These stations are described as follows:

Alamogordo. About three miles south of the town of Alamogordo, and about two miles west of the foothills of the Sacramento Mountains. Three females and six males were trapped in mesquite association. The soil is pinkish gray in color, and there are no rocks in this area.

TABLE I

Shade of Soils Inhabited, as Shown by Means of Ten Tint Photometer Readings with Red Filter on Each Sample
Shade of Pelage of Adult Cactus-mice (*Peromyscus eremicus*), Standard Deviations, with Standard Errors.

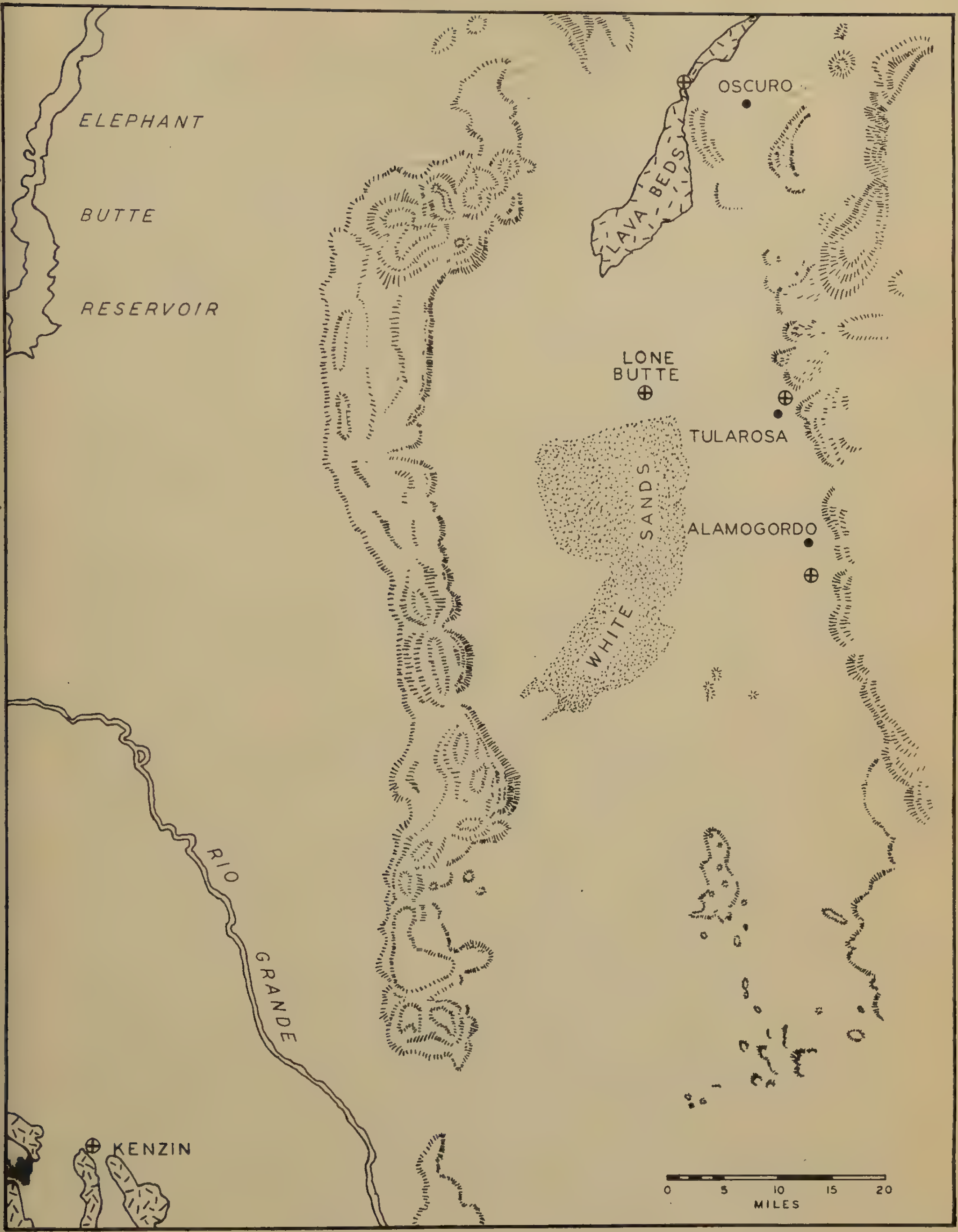
Stock	Shade of Soil Color	Specimens			
		Number Parents	Number Specimens	Means	Standard Deviations
Tularosa	12.3	9	42	14.52 ± 0.62	3.99 ± 0.44
Lone Butte	36.0	9	49	16.98 ± 0.35	2.42 ± 0.24
Oscuro	10.3	6	88	10.94 ± 0.24	2.21 ± 0.17
Kenzin	12.4	7	69	9.90 ± 0.25	2.07 ± 0.18
Alamogordo	19.6	9	39	17.80 ± 0.30	1.86 ± 0.21

Lone Butte. About thirteen miles west of Tularosa in a small, isolated area of mesquite association. Four females and five males were taken here. The soil is pale pinkish gray, and there are no rocks.

Tularosa. Three miles north of Tularosa, and about two miles west of the foothills of the mountains. Four females and five males were captured in grassy wash association. The soil is dark red, and there are no rocks.

Oscuro. The Tularosa Malpais about five miles west of Oscuro. Three males and three females were collected in Malpais lava association. The mice were living in crevices and holes in the black lava.

Kenzin. Lava bed at Kenzin, Dona Ana County. Three females and four males were obtained on the lava bed. The lava at this station is not quite as dark as the Tularosa Malpais lava (Table I).



Map 1. Tularosa Basin and adjoining areas in southern New Mexico, showing approximate location of stations at which cactus-mice were collected. Crossed circles indicate collecting stations.

SOIL COLORS

The two lava beds studied are composed of dark rock. The lava of the Tularosa Malpais is slightly darker than that of the Kenzin bed (Table I). Small quantities of soil paler than the lava rock have accumulated in pockets on both lava beds. This is particularly true of the Kenzin bed, where pale, orange-colored sand has been blown on the flattest part of the lava from near-by dunes. The cactus-mice live in crevices in the lava, but they undoubtedly travel over the pockets of soil as well as over the rough rock.

The soils of the Tularosa Basin are highly variable in shade. The creamy white gypsum of the White Sands, an area of approximately 270 square miles near the center of the basin, is the palest soil of the region. Pinkish gray soils, which occur over most of the basin, exclusive of the White Sands, are pale, but they do not approach the White Sands in this respect. Cactus-mice were collected at two stations on these pinkish gray soils. The soil at the Lone Butte station was paler than that at the Alamogordo station (Table I). An area of dark red soil occupies about twenty square miles of the desert just north of the town of Tularosa. This soil, while very different in hue, is as dark as the Kenzin lava. A stock of cactus-mice was taken on this dark, red soil. The soils in the foothills of the mountains along the eastern edge of the basin, are predominantly pale and pinkish gray. The Tularosa dark red soil extends for an undetermined distance into the foothills.

SHADE OF PELAGE COLOR

The colorimetric measurements of the field-caught and laboratory-raised cactus-mice are roughly indicative of the variation in shade of pelage in the respective wild populations. The parameters of the wild populations in respect to shade of pelage obviously cannot be estimated accurately from these data, for the numbers of field-caught parents are too small to afford fully reliable samples of the wild populations. Nevertheless, the laboratory stocks suffice to show the major trends in shade of pelage in the populations from which they were derived.

The Kenzin and Oscuro stocks, which are from dark lava beds, do not differ very greatly in shade of pelage (Table I). The Oscuro stock averages slightly, but significantly, paler than the Kenzin stock, the difference in the means is 1.04 ± 0.35 tint photometer units, which is significant. The lava-bed stocks are both significantly darker than any of the stocks from the desert plain.

The Tularosa stock, originating from a small area of dark red soil as dark in shade as the Kenzin lava, is intermediate in mean shade of pelage between the lava-bed stocks and the stocks from relatively pale, pinkish gray soils. The Tularosa stock is significantly paler than the lava-bed stocks and significantly darker than the Alamogordo and Lone Butte mice. The Tularosa stock is also significantly more variable, as shown by the standard deviations, than any of the other stock studied (Table I). The palest Tularosa animals are as pale as the palest of those from the other desert plain stocks, and the darkest approach the darkest of the lava-bed mice in depth of shade.

The Alamogordo and Lone Butte stocks, which are from relatively pale desert soils, are significantly paler than the three other stocks (Table I). There is no significant difference between these two stocks in mean shade of pelage, nor in variability, as shown by the standard deviations.

DISCUSSION

The tendency of desert mammals in general and of cactus-mice in particular to be inconspicuous on their backgrounds has been pointed out by Dice and Blossom (1932) and by Dice (1939). This tendency is evident in the cactus-mice of the Tularosa Basin, for they usually correspond in shade of pelage to that of the background on which they live. The soils of the basin form a mosaic of markedly different shades of color. The aggregate population of cactus-mice in the region, in turn, is a mosaic of a number of small, local populations that differ in shade of pelage. Local populations of similar shades of pelage occur on soils of similar shade many miles apart. Intervening areas of differently colored soils support populations with different shades of pelage. Thus, the dark races on the two widely separated, dark lava beds are similar in shade of pelage, while pale races occupy the intervening pale soils of the desert plain.

The differentiation of the local populations associated with local soil areas of different shades of color seems best attributable to selection pressure that exceeds the homogenizing effect of interbreeding between the several adjacent, local populations. The local distribution of the cactus-mouse is influenced by its preference for rocky situations, but, at some seasons of the year at least, it ranges more widely over the desert plains than any other primarily saxicolous mammal of the region (Blair, 1943). There is, therefore, incomplete ecological isolation of such populations as those on the two lava beds.

Even the small area of Tularosa red soil is large enough for differentiation in shade of pelage of cactus-mice to occur. Color differentiation of another species, *Peromyscus maniculatus,* on the Tularosa red soil and other soils of the desert plain has previously been reported (Blair, 1947). The Tularosa and Alamogordo stocks of cactus-mice are samples from a continuously distributed population that occupies the front and foothills of the Sacramento Mountains. There must be a fairly continuous interchange of individuals between the mice living on the dark red Tularosa soil and those living on the pinkish gray Alamogordo soil. The small size of the area of dark red soil at Tularosa and the swamping effect of gene dispersal from near-by areas of pinkish gray soils probably account for the relatively high variability of the Tularosa stock. Selection pressure and dispersal pressure evidently more or less balance one another. The selection pressure here seems insufficient to eliminate all or most of the genes making for paleness of shade. The influx of genes from surrounding areas seems insufficient to overcome completely the effects of selection against the genes for paleness.

An interchange of genes undoubtedly takes place between the populations of the Malpais lava and the populations of the near-by mountains, where the background is relatively pale. Benson (1933 : 41) has suggested that the variability of the Kenzin and Malpais lava populations of cactus-mice is due to immigration from surrounding territory. Such immigration and a consequent transfer of genes probably takes place between the other contiguous color populations of cactus-mice in the region. This interchange of genes, without the operation of some kind of selection, would theoretically result in a fairly homogeneous population of cactus-mice throughout the basin. The existence of these color races, therefore, can be explained adequately only as the result of natural selection.

SUMMARY

Cactus-mice in the Tularosa Basin of southern New Mexico usually correspond in shade of pelage to the shade of the background on which they live. Stocks from the Malpais lava in the northern part of the basin and from the Kenzin lava south of the basin are very similar to one another in mean shade of pelage. Relatively pale stocks were taken at Alamogordo and Lone Butte on the pinkish gray soils of the intervening desert basin. The Tularosa stock, which is from a relatively small area of dark red soil is intermediate in shade of pelage between the lava-bed stocks and the stocks from pinkish gray soils. The Tularosa stock, being from a relatively small area of uniform soil color, is more variable than are the other stocks. The existence of the local cactus-mouse populations distinguished by shade of pelage is attributed to selection pressure that exceeds the homogenizing effect of interbreeding between the several adjacent local populations.

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THE OCCURRENCE OF BUFF AND GRAY PELAGE IN WIDELY SEPARATED GEOGRAPHIC RACES OF THE DEER-MOUSE (*PEROMYSCUS MANICULATUS*)

BY W. FRANK BLAIR

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INTRODUCTION

THE rate of evolution of animals in nature can be estimated accurately only when it becomes possible to express the variations of wild populations in terms of variations in gene frequencies. Such pioneer efforts as those of Sumner (1932) and Dice (1940) have convincingly demonstrated the enormous range of variability in natural populations of *Peromyscus.* Further progress toward an understanding of the causes for physical differences between local populations, however, requires a different approach. The mode of inheritance of characters that distinguish natural populations must be analyzed, and the genotypes of wild-living individuals must be determined. It is, in a sense, unfortunate that our knowledge of the so-called mutant characters, of little or obscure importance to the immediate survival of the animal in nature, far exceeds our information about characters that receive general expression in natural populations. A beginning has been made, however, toward an understanding of the genetic basis for these "wild-type" characters.

Recent studies of color variation in the deer-mouse of the subspecies *Peromyscus maniculatus blandus* have shown that the buff and gray color phases of this race are produced by Mendelian alleles (Dice, 1933; Blair, 1947*a*). Populations of this subspecies of deer-mouse in the Tularosa Basin, New Mexico, have been sampled, and the frequencies of the two alleles in local populations of that area have been estimated (Blair, 1947*b*).

The present report deals with the results of experiments designed to test for the occurrence of these color genes in other populations from distant parts of the range of *Peromyscus maniculatus*. It includes also an attempt to classify as buff or as gray the field-caught specimens of this species in the collection of the Laboratory of Vertebrate Biology.

ACKNOWLEDGMENT

This study was conducted at the Laboratory of Vertebrate Biology, University of Michigan, as part of its research program.

METHODS

All of the deer-mice discussed herein were kept under standard conditions in the Laboratory of Vertebrate Biology at Ann Arbor, and all were fed a standard ration. The hybrids produced in the crosses between the several strains of mice were raised to an age of five or more months, when they were prepared as flat skins. The shade of pelage of some of these hybrids has been measured with a Photovolt reflection meter, using a standard technique (Blair, 1947*a*). Classification into color types was made after the mice had been prepared as specimens.

The field-caught mice that were examined for the buff and gray characters were brought into the laboratory in past years to initiate stocks for variability studies. They have been prepared as flat skins and stored in museum cases. These mice represent random samples of the populations from which they were taken.

STRAINS OF DEER-MICE

Representatives of five strains of deer-mice, either field-caught mice or their direct descendants, have been used in the crosses with selected color strains of *blandus*. The origin of the stocks used is as follows:

Blandus, Tularosa. These color strains of *blandus* have been described in detail elsewhere (Blair, 1947*a*). Most of the *blandus* mice used in the crosses were homozygous for recessive gray (genotype $g\,g$). Most of these grays were also homozygous for another, dominant gene, black-tipped (genotype $B\,B$), but, as will be indicated in the text, a few heterozygotes ($B\,b$) and a few mice that were homozygous for recessive brown-tipped ($b\,b$) were also used. Black-tipped buffs ($BB\,GG$) were crossed with two of the stocks from other localities.

Nebrascensis, Meade. This stock originated from six females and seven males trapped by C. W. Hibbard about fourteen miles southwest of Meade, Meade County, Kansas. The field-caught mice were received in the laboratory on August 22, 1939.

Gracilis, Pine Lake. These mice descended from eight males and eight females that were trapped by W. F. Blair near Pine Lake, Alger County, Michigan. The field-caught mice reached the laboratory on September 24, 1940.

Nubiterrae, Mountain Lake. Fourteen males and five females were collected by J. S. Rogers, Jr., near Mountain Lake, Virginia. They were received on July 22, 1939.

Oreas, Oyster Bay. This stock originated from nine males and six females that were taken by H. J. Leraas on Oyster Bay, about ten miles west of Olympia, Washington. The field-caught mice were received in the laboratory on December 13, 1940.

Oreas, Silver Lake. Nine males and two females were trapped, July 18 and 19, 1940, by Lee R. Dice about five miles west of Eatonville, Pierce County, Washington.

CROSSES BETWEEN *blandus* COLOR TYPES AND OTHER GEOGRAPHICALLY DISTANT RACES

Crosses were made between certain of the *blandus* color strains and deer-mice from the five distant localities described above. These mice from other localities are fairly representative of the species *maniculatus.* Two stocks are from the Pacific Northwest. One is from the southern range of the species in the Eastern forest. One is from the northern part of the range in the Eastern forest. Another is from the central Great Plains.

The crosses were made to test the hypotheses that (1) the buff character of other races of deer-mice results from the same major gene that produces buff *blandus,* and that (2) all gray deer-mice have the recessive gene for gray in common. The results of the crosses are summarized in Tables I and II. The segregation of color characters in the crosses is discussed below.

Gray *blandus* × Buff *nebrascensis*

The *nebrascensis* deer-mice, from southwestern Kansas, are a rich buff in pelage. Ten field-caught mice have been examined, and all are buff, although there is some variation in the saturation of yellow-orange pigment. Two of these buff *nebrascensis* were crossed with gray *blandus.* The two matings produced seventeen F_1 mice, all of which were buff. This indicates that the buff of the *nebrascensis* dominates the *blandus* gray. Although all F_1 mice were buff, there was much variation in shade and saturation of color. One of the matings, between a bright buff *nebrascensis* and a gray *blandus,* produced five bright buff offspring. The other mating, between a dull buff *nebrascensis* and a gray *blandus,* produced a variable lot of twelve dull buff mice.

Four pairs of the F_1 mice produced an F_2 generation of sixty-six buffs and twenty-seven grays (Table I). The ratio of buffs to grays does not depart significantly from the expected three to one ratio for a simple Mendelian cross. The gray segregants were pale and did not differ in appearance from pale gray *blandus.* This is taken to indicate that the *nebrascensis* mice contributed no appreciable quantity of modifiers of gray to the cross. The buff segregants were variable in the shade and saturation of color. Each sibship contained some bright buff mice and some mice which, presumably due to the segregation of minor modifying genes, were of variable shades and saturations of buff.

Seven of the F_1 mice, mated back to gray *blandus,* produced a backcross generation of forty-four buff and thirty-seven gray mice (Table II). The buff–gray ratio does not differ significantly from the expected one to one ratio for a simple backcross. The grays were pale in shade and similar to gray *blandus* in appearance. The buffs, like the F_2 buffs, were variable in shade and saturation of color. One sibship contained one brown-tipped buff and one brown-tipped gray. The brown-tipped presumably came from the *blandus.* The brown-tipped mice were of medium shade and were similar in appearance to *blandus* mice of corresponding phenotype.

TABLE I

SEGREGATION OF BUFF AND GRAY PHENOTYPES IN CROSSES BETWEEN *blandus,* TULAROSA, NEW MEXICO, COLOR STRAINS AND DEER-MICE FROM OTHER, DISTANT LOCALITIES

Crosses	F_1			F_2			
	Expected Number	Phenotype	Observed Number	Expected Number	Classes of Phenotypes	Observed Number	χ^2
Gray *blandus* × buff *nebrascensis*—Meade	17	Buff	17	69.75	Buff	66	0.806
				23.25	Gray	27	
Buff *blandus* × buff *nebrascensis*—Meade	17	Buff	17	41.00	Buff	41	....
Gray *blandus* × buff *gracilis*—Pine Lake	7	Buff	7	2.25	Buff	2	....
				0.75	Gray	1	
Buff *blandus* × buff *gracilis*—Pine Lake	8	Buff	8	....	....	......	....
Gray *blandus* × buff *nubiterrae*—Mt. Lake	51	Buff	44	6.00	Buff	4	....
		Gray	7	2.00	Gray	4	
Gray *blandus* × gray *nubiterrae*—Mt. Lake	9	Gray	9	8.00	Gray	8	....
Gray *blandus* × buff *oreas*—Oyster Bay	6	Buff	6	10.50	Buff	10	....
				3.50	Gray	4	
Gray *blandus* × buff *oreas*—Silver Lake	8	Buff	8	13.50	Buff	13	....
				4.50	Gray	5	

The dominance of the *nebrascensis* buff over the *blandus* gray in the F_1 and the segregation in the F_2 and backcross generations indicates that these are allelic genes and that the buff dominates the gray. The similarity in appearance between the *nebrascensis* buff and the *blandus* buff, and the results of the cross to be described below, indicate that the same major gene for buff pelage is common to the *blandus* buffs and the *nebrascensis* buffs, at least to those from the Meade locality.

Buff *blandus* × Buff *nebrascensis*

Two of the buff *nebrascensis* were crossed with black-tipped buff *blandus*. One mating produced six and the other produced eleven buff offspring. Five members of one sibship, which were prepared as specimens, had an intense saturation of yellow-orange, but they varied among themselves considerably in shade of pelage. Four pairs of these F_1 mice were mated and produced an F_2 generation of forty-one buff mice (Table I). These mice showed the same sort of variability in shade and saturation of pelage color, presumably due to the segregation of minor modifying genes, that was present in the buffs of the F_2 and backcross generations of the cross between gray *blandus* and buff *nebrascensis*. There was no other evident segregation. It seems evident, then, that the *blandus* and *nebrascensis* buffs are produced by the same dominant gene for buff pelage.

Gray *blandus* × Buff *gracilis*

The *gracilis* stock, from the Upper Peninsula of Michigan, descended from seventeen dark buff, field-caught mice. Two of the buff *gracilis*, crossed with gray *blandus*, produced seven moderately bright buffs in the F_1 generation. A mating between two of the F_1 mice resulted in an F_2 generation of

TABLE II

SEGREGATION OF COLOR PHENOTYPE IN BACKCROSSES OF HYBRIDS (BETWEEN *blandus*-GRAY AND BUFF DEER-MICE FROM OTHER LOCALITIES) TO *blandus*-GRAY

Backcrosses	Expected Number	Classes of Phenotypes	Observed Number	χ^2
F_1 (gray *blandus* × buff *nebrascensis*) ×	40.50	Buff	44	0.605
gray *blandus*	40.50	Gray	37	
F_1 (gray *blandus* × buff *gracilis*) ×	35.00	Buff	30	1.429
gray *blandus*	35.00	Gray	40	
F_1 (gray *blandus* × buff *nubiterrae*) ×	14.00	Buff	16	0.571
gray *blandus*	14.00	Gray	12	
F_1 (gray *blandus* × buff *oreas*—Oyster Bay) ×	37.50	Buff	42	1.080
gray *blandus*	37.50	Gray	33	
F_1 (gray *blandus* × buff *oreas*—Silver Lake) ×	40.00	Buff	38	0.200
gray *blandus*	40.00	Gray	42	

one gray and two buff mice (Table I). The gray was no different from that of gray *blandus* mice. One buff was moderately bright; the other was dull in appearance and had a low intensity of yellow-orange pigment.

Four of the F_1 mice were backcrossed to gray *blandus* and produced thirty buff and forty gray offspring (Table II). The ratio of buffs to grays does not depart significantly from the expected one to one ratio for a simple backcross. The grays did not differ in appearance from gray *blandus* mice. The buffs were highly variable. Some were bright, with a high intensity of yellow-orange; others were dull, with a low intensity of yellow-orange.

The gray *blandus* used in one of the original and backcross matings was in the brown-tipped phase. The backcross generation, in this case, contained six black-tipped buffs, six black-tipped grays, six brown-tipped buffs, and three brown-tipped grays. The brown-tipped buffs and grays resembled corresponding phenotypes, of average shade, in *blandus*. The results of the crosses between *blandus* and *gracilis* indicate that the buff of *gracilis* is an allele of the gray of *blandus*. The similarity of the hybrid buffs, in both the black-tipped and brown-tipped phases, to corresponding *blandus* types strongly suggests that the *blandus* buff and the *gracilis* buff are both produced by the same major color gene. The buff of both subspecies dominates gray, which furnishes additional cause to believe that the buff of both results from the same gene.

Buff *blandus* × Buff *gracilis*

Three of the buff *gracilis* crossed with buff *blandus* produced an F_1 generation of eight buffs. The hybrids were extremely variable in their saturation of yellow-orange pigment. One sibship contained only one mouse, which was dull buff and had a medium saturation of yellow-orange. Another sibship had one bright buff, one of medium brightness, and one dull buff that had a low saturation of yellow-orange. The variability of the buff hybrids presumably comes from various combinations of several minor modifying genes that affect the intensity of the yellow-orange pigment.

Gray *blandus* × Buff *oreas*—Oyster Bay

Four field-caught *oreas* from Puget Sound, Washington, are dark in shade and buffy in tone. The yellow-orange pigment in these mice is of medium or low intensity. One of the buff *oreas* mice was crossed with a gray *blandus*, and the mating produced six dull buff offspring. There was little variability in shade of pelage or in amount of yellow-orange saturation in this family of six hybrids. Two mated pairs of the F_1 mice produced an F_2 generation of ten buffs and four grays. The buff–gray ratio is close to the expected three to one ratio (Table I). The grays resembled dark gray *blandus*. The buffs in both families of F_2 hybrids were extremely variable, which presumably indicates numerous minor modifiers of buff in the *oreas*. Each family had at least one fairly bright buff and also intermediates between bright buff and dull buff. Two mice of one family were a "chocolate" buff in appearance, and one mouse of the other family was of this type.

Four of the F_1 hybrids were backcrossed to gray *blandus.* The backcross generation comprised forty-two buffs and thirty-three grays, which is within the limits of variability for the expected one to one ratio (Table II). The gray *blandus* used in the original cross and for the backcross were heterozygous for brown-tipped; thus, the segregation in three sibships of the backcross generation was into four classes. The black-tipped and brown-tipped grays resembled corresponding phases of gray *blandus.* The black-tipped buffs were less variable than the F_2 buffs and were low in yellow-orange saturation. The buff of *oreas,* like the buff in the other races, appears to result from the same gene that produces the buff *blandus.* The differences in appearance of the two buffs apparently are caused by several modifying genes.

Gray *blandus* × Buff *oreas*—Silver Lake

The eleven field-caught *oreas* from Silver Lake, Washington, resembled the *oreas* from Oyster Bay. One Silver Lake *oreas* was crossed with a gray *blandus.* Eight buff offspring resulted from this cross. These buffs were variable in shade of pelage and ranged from moderate to low in saturation of yellow-orange. An F_2 generation, from four pairs of the hybrids, comprised thirteen buffs and five grays. The buff–gray ratio is within the limits of probability for the three to one ratio that would be expected if this buff is a single unit dominant over *blandus* gray. The grays, two of which were in the brown-tipped phase, resembled corresponding phases of dark gray *blandus.* The buffs were variable. Two had moderate intensities of yellow-orange pigment; the others were low in intensity of this pigment. There was much variation in general shade of pelage.

Seven of the F_1 hybrids were backcrossed to gray *blandus.* Their progeny comprised thirty-eight buffs and forty grays. The proportions of buffs and grays are close to the expected one to one ratio (Table II). The grays resembled gray *blandus.* The buffs varied in shade and in saturation of yellow-orange pigment. One sibship contained three buffs in the brown-tipped phase. Two of these resembled brown-tipped buff *blandus.* The other one was, presumably from the action of modifying genes, more dull and more brown than the corresponding type of *blandus.* The buff of this *oreas* stock, like that of the *oreas* from Oyster Bay, therefore, appears to be produced by the same major gene that produces buff *blandus.*

Gray *blandus* × Buff *nubiterrae*

Fourteen of seventeen field-caught *nubiterrae* from Mountain Lake, Virginia, were fairly bright buff in appearance. Six apparently buff offspring of the field-caught mice were crossed with gray *blandus.* Three of the ensuing sibships contained an aggregate of twenty-four variable buffs. One was very bright buff, four were moderately bright buff, and nineteen were a dull "chocolate" buff similar to the "chocolate" buffs among the Oyster Bay *oreas* hybrids. Three sibships comprised twenty-one buffs, which showed the same type of variability as was present in the families mentioned above, and five apparent grays. These suspected grays were more brown in appearance than gray *blandus.*

Two pairs of buff hybrids produced an F_2 generation of four variable, but relatively bright, buffs and four variable grays. One of the grays resembled dark gray *blandus;* the others were more chocolate-brown in appearance than were the gray *blandus* mice.

Five of the buff F_1 mice were backcrossed to gray *blandus.* The backcross generation comprised sixteen variable buffs and twelve grays. The buff–gray ratio does not depart significantly from the expected one to one ratio. The gray segregants in this cross, unlike those in the crosses involving other stocks, differed from gray *blandus.* These hybrid grays were all more brown in appearance than were the gray *blandus* mice. It appears that the *nubiterrae* mice possessed certain modifiers of gray that were not observed in the crosses involving other stocks. The evidence from this cross suggests that the doubtful grays that segregated out in the F_1 generation of the cross were grays. Like the grays in this generation, they were more brown in appearance than were the gray *blandus.*

Gray *blandus* × Gray *nubiterrae*

Three of the seventeen field-caught *nubiterrae* were darker in shade and more gray in appearance than the others. These three mice were thought to be possibly gray rather than buff, although they had low intensities of what appeared to be yellow-orange pigment. One of these mice was mated to a gray *blandus.* The nine offspring of this mating were gray. One resembled dark gray *blandus,* and the others were slightly more brown than *blandus.* Two of these brownish gray hybrids, when mated together, produced an F_2 generation of eight grays. Three of the grays resembled dark gray *blandus,* and the others were more brown than the *blandus* mice were.

The modification in tone of the *blandus* gray, when crossed with *nubiterrae,* evidently results from the action of one or more modifying genes. This effect is quite different from the intensification of the shade of gray that is obtained when pale gray *blandus* are crossed with other deer-mice that have a dark shade of pelage. If I am correct in believing that the gray of *nubiterrae* and of *blandus* is produced by the same major gene, with different modifiers, then the *nubiterrae* stock comes from a population having a mixture of buff and gray genes.

BUFF AND GRAY PHASES IN SAMPLES OF WILD POPULATIONS

All field-caught specimens of *Peromyscus maniculatus* in the collection of the Laboratory of Vertebrate Biology have been examined for the buff–gray pelage characters. These specimens total 2467 and represent eighteen named races of deer-mice from 173 localities in North America. Four regions, the Great Lakes states, northern plains, northwestern United States, and the southwestern deserts, are most heavily represented. Some specimens are available, however, from most parts of the range of the species in the United States.

Not all of the mice can be classified positively as buffs or grays. Modifiers of gray, such as were present in the *nubiterrae* stock that was crossed with gray *blandus,* are apparently widespread in the species. The buffs are extremely variable in their intensity of yellow-orange pigment and in their shade of pelage. Certain intensifiers of shade of pelage often suppress buffiness in mice that are genotypically buff (Blair, 1947*a*). It is not possible, therefore, to classify all deer-mice in respect to buff and gray without breeding tests.

Of the 2467 specimens examined, 2089 are classified as buff. These come from 172 localities and represent eighteen subspecies. Many of these buffs are more brown, or chocolate, in appearance than is buff *blandus.* Grays of the *blandus* type total 177, but 158 of these are from the Tularosa Basin, New Mexico. Two other stocks of *blandus,* from the Davis Mountains of Texas, contain grays of this type. One of three specimens of *rufinus* from Highrolls in the Sacramento Mountains, New Mexico, resembles the gray *blandus.* Two of twenty-three *artemisiae* from Troy, Idaho, resemble gray *blandus.* One of 139 *maniculatus* from Isle Royale in Lake Superior is indistinguishable from dark gray *blandus.*

Seventy-five of the mice examined are possibly gray. They are more brown in appearance than are gray *blandus* mice, and like the *nubiterrae* grays used in the *blandus–nubiterrae* cross, they appear to have low intensities of yellow-orange pigment. Breeding tests would be required to determine whether such mice were buff or gray. Such a test did indicate that the *nubiterrae* mice of this type are modified grays rather than buffs. These presumed grays were mostly in samples from populations with dark shades of pelage. Stocks of *hollisteri, rubidus,* and *artemisiae* from the forests of the Northwest and of *maniculatus* and *nubiterrae* from the Eastern forests contained mice of this type. Other specimens of this possible gray were found in the dark-colored race *bairdii* of the prairies and in *osgoodi* and *gambeli.* It is noteworthy that two of the stocks in which grays resembling gray *blandus* were present contained many of these other, supposed grays. The stock of *artemisiae* from Troy, Idaho, in addition to two grays of *blandus* type, contained nine of this other type and twelve buffs. The stock of *maniculatus* from Isle Royale, Michigan, in addition to one *blandus*-type gray, contained thirty-two mice that were classified as the modified gray and 106 that were classified as buffs or possible buffs.

There are 117 specimens that, while low in intensity of yellow-orange pigment, appear to be probably buff rather than gray. These mice could be classified with certainty only by a breeding test.

Some generalizations may be made from the examination of the field-caught specimens even though it is impossible to classify all individuals in respect to color type. It is very evident that most deer-mice are buff.

Gray deer-mice most frequently inhabit the Southwestern deserts, but some grays do occur in other distant populations. Grays that resemble gray *blandus* rarely are present in these other populations. Brownish gray mice that possibly are modifications of the gray observed in *blandus,* presumably through the effects of other genes, occur in several widely separated populations of the species.

Deer-mice of the subspecies *nebrascensis* from the Nebraska sandhills have a type of dichromatism. An aggregate of 166 specimens from eighteen localities in and near the sandhills was examined, and all of the mice appear to be of the buff phase. Of these, 124 mice are a clear, rich yellow-orange in appearance, and forty-two are relatively dull in appearance and dark in shade of pelage. Clark (1938) crossed some of these clear buff *nebrascensis* with dark-colored mice of the subspecies *bairdii* and *rubidus,* which he called gray. From these crosses he obtained evidence that the *nebrascensis* character is dominant over the dark shade of pelage of *bairdii* and *oreas.* His material has been examined by me, and it is evident that the characters he was dealing with do not correspond to the buff and gray of *blandus,* for all of his mice are buffs by my classification. The relating of these characters to other described color characters of the deer-mouse must await further investigation.

DISCUSSION

The cross matings showed that the gray of *blandus* is recessive to the buff of (1) *nebrascensis* from southwestern Kansas, (2) *gracilis* from northern Michigan, (3) *nubiterrae* from Virginia, and (4) two stocks of *oreas* from the state of Washington. The resemblance of buff *blandus* to the buff segregants in the crosses and the lack of evident segregation in the F_2 of the cross between buff *blandus* and buff *nebrascensis* indicate that the buff of the other races of *maniculatus* is probably produced by the same major gene that produces buff *blandus.*

The *nubiterrae* stock was the only one of the five stocks tested that had both of the buff–gray alleles. Failure to find the recessive gray gene in the other races is no indication that these races lack the gene. Too few individuals were tested for any conclusions about the frequencies of the two genes to be drawn from these data. Actually, gray mice resembling gray *blandus* were observed in samples from three other subspecies, and brownish gray mice, presumably produced by the gene for gray and other, modifying genes, were among field-caught specimens from eight geographic races.

The buff segregants in the crosses between the *blandus* color strains and the other races are extremely variable. Some are bright buff in appearance; others are dull buff. The buffs vary extremely in the intensity of the yellow-orange pigment in the agouti band of the body hairs. The wide range of variation in the hybrid buffs is taken to indicate numerous modifying genes with minor effects on the buff phenotype. Similar effects of these minor genes have been observed in crosses between color strains of *blandus* (Blair, 1947*a*). The buff field-caught specimens examined from many parts of the species range vary similarly. The variability is so great that, lacking the opportunity for breeding tests, not all of these field-caught mice can be definitely classified as buffs.

The gray segregants in the crosses between gray *blandus* and the *oreas, gracilis,* and *nebrascensis* show very little variation. The hybrid grays resemble gray *blandus* of corresponding shades of pelage. The *blandus* gray is darkened by the cross to the relatively dark races of deer-mice, just as it can be darkened by crossing a pale with a dark gray *blandus*. The cross with *oreas,* which, in shade of pelage, is the darkest of the races studied, produces the darkest shade of grays (Table III). The grays in the backcross generation of the crosses are paler than those of the F_2 generation. The minor modifiers of the intensity of shade are presumably reduced in number by the backcross to gray *blandus*. The genes that modify the shade of gray in these other races, since their effects are similar, are possibly the same genes that modify the shade of gray in *blandus* populations.

TABLE III

MEAN SHADE OF GRAY MICE IN F_2 AND BACKCROSS GENERATIONS OF CROSSES BETWEEN GRAY *blandus* AND MICE OF OTHER RACES, AS SHOWN BY REFLECTION METER READINGS FOR DORSAL RED

Cross	Generation			
	F_2		Backcross	
	Specimens	Dorsal Red	Specimens	Dorsal Red
Gray *blandus* × buff *oreas* (O.B.)	4	12.50	24	16.08 ± 0.41
Gray *blandus* × buff *oreas* (S.L.)	3	13.00	41	15.46 ± 0.32
Gray *blandus* × buff *nebrascensis*	27	16.74 ± 0.39	44	18.41 ± 0.33
Gray *blandus* × buff *gracilis*	1	19.00	37	19.03 ± 0.36
Gray *blandus* × buff *nubiterrae*	5	15.40	12	18.17 ± 0.45
Gray *blandus* × gray *nubiterrae*	8	13.50	......	....

Gray segregants in crosses between gray *blandus* and mice of the subspecies *nubiterrae* are usually more brown in appearance than are gray *blandus* mice. One gray segregant in a cross between *oreas* and gray *blandus* was of this type. This brown effect apparently results from the effects of one or more other color genes. The mode of inheritance of these other genes and their relations to previously described color genes of the deer-mouse have yet to be determined. The brown effect here is not produced by the previously described (Blair, 1947*a*) gene—brown-tipped. A "chocolate" buff was observed in some of the segregants in crosses between *nubiterrae* and *blandus* and between *oreas* and *blandus*. This "chocolate" buff is presumably caused by the same gene or genes that produce the brownish gray observed in those races. Some individuals in eight of the eighteen subspecies represented by field-caught samples are of the brownish gray type. Some of the field-caught buffs, likewise, are "chocolate" buff in appearance. It is evident, therefore, that the gene or genes for this brown character are widely distributed in deer-mouse populations.

SUMMARY AND CONCLUSIONS

This report deals principally with the distribution of the buff and the gray pelage color alleles (*G, g*) in populations of the deer-mouse (*Peromyscus maniculatus*). Representatives of four subspecies, *nebrascensis, gracilis, nubiterrae,* and *oreas,* were crossed with gray *blandus*. The buff of these other races is dominant over the *blandus* gray. The appearance of the buff hybrids of these crosses and the lack of evident segregation in crosses between buff *blandus* and buff *nebrascensis* and *gracilis* indicate that the same major gene for buff probably is present in all of the races of *maniculatus.*

Buff segregants in the crosses between *blandus* and the other races are extremely variable in shade and in the intensity of yellow-orange pigment in the agouti band of the hairs. This extreme variability of the buffs presumably results from the action of numerous minor modifying genes. Some of the buffs appear to be "chocolate" buff, presumably from the effects of other color genes. Gray segregants are less variable than the buffs, but some of the grays are more brown in appearance than is gray *blandus*. The brownishness of some individuals of both the buffs and grays presumably is caused by the same, as yet undetermined, gene or genes.

All field-caught specimens of deer-mice in the collection of the Laboratory of Vertebrate Biology were examined, and an attempt was made to classify each specimen as buff or gray. Of the 2467 specimens examined, 2089 are classified as buff, and 117 as possibly buff. A total of 177, of which 158 are from the Tularosa Basin, New Mexico, are classified as *blandus*-type gray. Seventy-five specimens are more brown in appearance than is gray *blandus*. These probably are grays modified by other genes.

This preliminary survey of the genetic basis for color variation in the deer-mouse serves principally to suggest possible future lines of attack on the problem of color differentiation in this species. The buff and gray genes, originally studied in *blandus* from New Mexico, are indicated to have wide distribution in the species *maniculatus.* No other members of this allelic series have yet been discovered. Most deer-mice are buff except in the Southwestern deserts, where high frequencies of the gray gene occur in some local populations (Blair, 1947*b*). The frequencies of the buff and gray genes now can be determined in other populations of the species by test matings between representatives of the wild populations and established laboratory strains of the *blandus* color types.

The color differentiation of the dark forest races of the Pacific Northwest and the Eastern forest and of the dark prairie race, *bairdii,* appears to be due in part to the effects of a gene or genes giving a brown tone to the pelage. The mode of inheritance of this character has yet to be determined.

A rich clear buff pelage character predominates in the sandhills of Nebraska. This character has been shown to be a single unit dominant over the dark pelage of such races as *bairdii* and *rubidus.* These alleles seem to be different from the other reported color genes of the deer-mouse, and they need further investigation and description.

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CELLULAR ANTIGENS IN THREE STOCKS OF *PEROMYSCUS MANICULATUS* FROM THE COLUMBIA RIVER VALLEY

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INTRODUCTION

ONE stock of *Peromyscus maniculatus* from the north side of the Columbia River and two stocks from the south side of the river were investigated (1) to determine the occurrence and nature of intraspecific differences in the red blood cell antigens, and (2) to test the utility of the red cell agglutination reaction as an auxiliary to morphological investigations of the interrelationships of microgeographic races.

Antigenic differences between species within the genus *Peromyscus* have been demonstrated. Moody (1941) found such differences between the cells of *P. leucopus* and those of *P. maniculatus*. Cotterman (1944) employed normal human group A sera to differentiate a series of species of *Peromyscus* into two groups: one group, which included *maniculatus,* possessing an antigen similar to the human isoagglutinogen B, the other group lacking this antigen. Absorption of the sera with *P. polionotus* cells removed all antibodies capable of reacting with the latter but left agglutinins for cells of the closely related species *maniculatus* and of F_1 *maniculatus* × *polionotus* hybrids. Absorption of the sera with *maniculatus* cells removed agglutinins for cells of both species and of the hybrids. These results indicate antigenic differences between species of sufficiently close relationship so that ready hybridization occurs.

Similar tests of antigenic differences at the subspecific and infra-subspecific levels in *Peromyscus* appear not to have been made. Employing strains of laboratory mice (presumably varieties of *Mus musculus*) Gorer (1936*a*) distinguished with human group A sera two antigenic types and demonstrated that the difference depended upon a single gene. The same author (1936*b*; 1937) explored the antigenic constitution of three inbred strains of laboratory mice, employing immune sera much after the manner of the present investigation. He identified four antigenic factors variably distributed among the strains of mice investigated (*vide infra*).

ACKNOWLEDGMENTS

This study was conducted at the University of Vermont. I am grateful to Lee R. Dice and Harold J. Leraas for obtaining the ancestors of the stocks utilized. These mice were furnished by the Laboratory of Vertebrate Biology, University of Michigan. I am indebted to Dr. Dice for counsel in connection with the writing of the paper.

MATERIALS AND METHODS

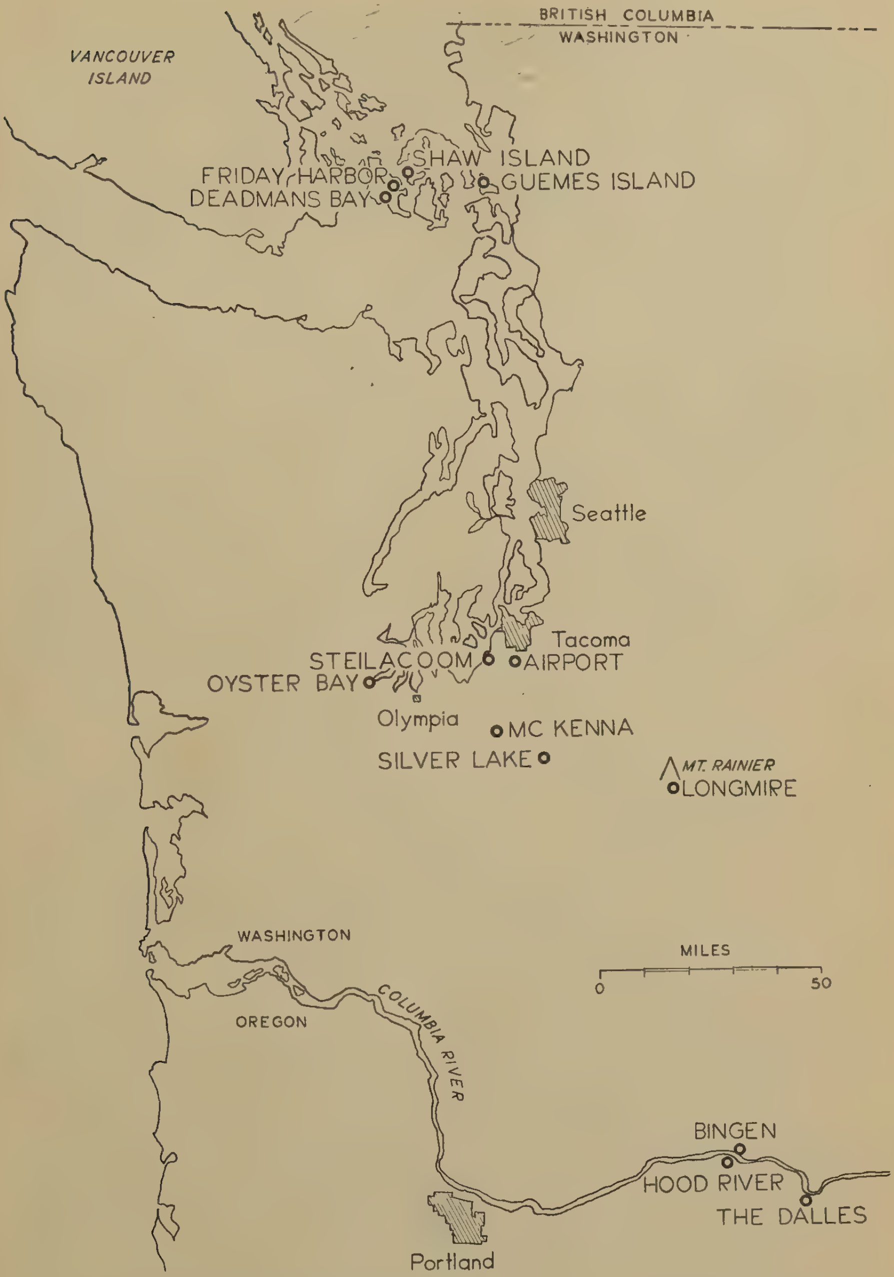
Stocks of Deer-Mice Included in the Study

The ancestors of three of the stocks employed were collected by Lee R. Dice during the summer of 1940. The stocks will be designated by the name of the locality from which each came. The three stocks from the Columbia River Valley originated, respectively, near the cities of The Dalles, Oregon, Hood River, Oregon, and Bingen, Washington. It will be noted from Map 1 that these localities are rather closely grouped geographically. Bingen and Hood River are almost directly across the river from each other; The Dalles is some twenty miles east of and on the same side of the Columbia River as is Hood River. Interest attaches to the question of whether or not the Columbia River here may form a barrier to the dispersal of deer-mice.

Dice (personal communication) has tentatively identified the Dalles and Bingen stocks as *P. maniculatus gambeli*. The Hood River stock is somewhat intermediate between *gambeli* and *rubidus*, but the larger number of characters points toward the latter subspecies rather than the former.

The Oyster Bay stock was collected in 1940 by Harold J. Leraas at a locality ten miles west of Olympia, Washington, on Oyster Bay. This stock is referred tentatively to *P. maniculatus austerus.*

Most of the mice utilized in the investigation were second and third generation descendants of the wild-caught animals. Although the stocks were not deliberately inbred, they were composed of the descendents of a relatively small number of individuals. Thus, it was not possible to be sure that each stock represented a truly random sample of the genotypes prevalent in the locality from which the stock was derived.



MAP 1. Western Washington and adjacent parts of Oregon, with localities from which the stocks of deer-mice originated.

Antiserum

The antiserum utilized was produced by inoculation of a rabbit with a 30 per cent suspension of pooled blood cells from mice of the Dalles stock. A total of 16.45 ml. of the cell suspension was inoculated intravenously in three six-day series. Blood was collected by cardiac puncture on the twelfth day following the last injection. The blood was defibrinated, and the serum was stored in sealed ampoules in the refrigerator. Technique was described by Moody (1941).

Absorption of Antiserum

Cells were collected from the mice by cardiac puncture, citrated, and washed three times with sterile 0.9 per cent buffered sodium chloride solution (cf. Moody, 1941). The blood from several mice was pooled, the number depending in part upon the quantity of antiserum to be absorbed.

The antiserum was diluted 1:100. Ten parts of diluted antiserum were usually added to one part of packed cells, although in final absorptions a proportion of 5:1 was frequently employed. Incubation was usually at room temperature for one or two hours, with frequent agitation. Occasionally, incubation at 37.5° C. for one hour was employed, though with no apparent difference in effect. At least two successive absorptions were required to remove completely antibodies capable of agglutinating a given type of cell.

Tests with the Absorbed Antiserum

Cells were collected as previously described and washed twice. They were used the day collected or the day following, being kept in the refrigerator meanwhile, to ensure against loss of sensitivity.

Tests were performed in 7 mm. test tubes using a modification of the method described by Boyd (1943: 360). Into each tube was pipetted 0.05 ml. of absorbed antiserum. To this was added a tiny loopful (a loop of No. 23 gauge Chromel wire made as small as possible without closing the opening) of the packed cells. Each tube was accompanied by a control containing 0.05 ml. of the physiological saline solution and a loopful of packed cells. The tubes were shaken, incubated for five minutes at room temperature with frequent additional shaking, and centrifuged for three minutes at approximately 2000 r.p.m.

The tests were read immediately. Macroscopic agglutination was observed by shaking the tubes gently until the cells in the control tubes became uniformly suspended. If cells in a test tube remained in a consolidated pellet the symbol "++++" was recorded; coarse flocculation was designated "+++," fine flocculation visible to the unaided eye, "++." Final readings were made with a binocular dissecting microscope employing 9 × oculars and 3.4 × objective. The stage was lighted from below with a substage microscope lamp. The tube to be read was placed on the stage in a holder which held the tube in a nearly horizontal position above a slit through which the light shone. Before being placed in the holder the tube was tilted so that the contents flowed toward the open end. The cells were viewed as they flowed back toward the closed end, the tube being in position in the holder above the lighted slit. Experience demonstrated that traces of agglutination could not be detected with certainty unless the cells were moving. If under the conditions described the agglutination had a granular appearance the symbol "+" was recorded. If the clumps were smaller and fewer the symbol "±" was used. In each case the tube containing antiserum was compared with the corresponding control tube. Traces of clotting sometimes simulated agglutination; error in this regard was avoided by observation of the control tube.

Apparently nature abhors an "endpoint." Reactions approach zero asymptotically. Agglutinations designated as "+" or "±" were definite and undoubted reactions, but between "±" and "–" a finely graded series of intermediate traces of agglutination was frequent. Despite my best efforts at objectivity, subjective errors in reading the tests undoubtedly occurred. In the interpretation of results faint traces of reaction were recorded as negative; only reactions of at least sufficient magnitude to be designated "±" were considered positive. Decision to adopt this criterion was influenced in part by the fact that at times faint traces of agglutination for the cells of a given individual remained even after such cells had been used twice in absorption of the antiserum. Apparently, absorption with pooled cells may not in every case remove all antibodies capable of reacting with the cells of every individual contributing to the pool. It seems, however, that this source of error, inherent in the use of pooled cells, was not significant in the present instance.

Absorbed Reagents

The diluted antiserum was first absorbed with cells of the Oyster Bay stock (*P. m. austerus*) to remove all antibodies for antigens shared in common by all members of the species *Peromyscus maniculatus*. Two successive absorptions, one incubated at room temperature the other at 37.5° C., were employed. In the first absorption macroscopic agglutination was immediate and heavy, seemingly involving the greater proportion of antibody content of the antiserum (Fig. 1).

Following absorption with cells of the Oyster Bay stock each reagent received subsequent additional absorption as described below.

RESULTS

REAGENT I. This reagent was prepared by absorbing diluted antiserum with cells from the Oyster Bay stock, as described above, and then with cells from selected individuals of the Dalles stock. Cells of the individuals selected had been found in preliminary tests to be definitely but submaximally agglutinated by a sample of the antiserum absorbed with Oyster Bay cells and partly absorbed with Dalles cells.

Absorption of reagent I was continued until all antibodies capable of reacting with cells of all surviving Dalles individuals had been removed. Four successive absorptions were necessary. The reagent then contained antibodies capable of agglutinating cells of eight of twenty-nine Bingen mice tested, and of six of thirty-three Hood River mice tested (Table I). The antigenic component with which the residual antibodies in reagent I reacted is designated BH_2.

Reagent II. After preliminary absorption with cells of Oyster Bay mice, this sample of diluted antiserum was absorbed with Bingen cells which had been found in preliminary tests to be definitely but submaximally agglutinated by a sample of the antiserum absorbed with Oyster Bay cells and partly absorbed with Bingen cells. In later absorptions of reagent II cells which gave stronger reactions in the preliminary tests were used. Five successive absorptions removed all antibodies capable of reacting with cells of any surviving members of the Bingen stock.

The reagent then contained antibodies capable of agglutinating cells of ten of nineteen Dalles mice tested, and of five of nineteen Hood River mice tested (Table I). The antigenic component with which the residual antibodies in reagent II reacted is designated DH_2.

Reagent III. After preliminary absorption with Oyster Bay cells, this sample of antiserum was absorbed with Dalles cells found to contain DH_2 by tests with reagent II. Three absorptions removed all antibodies capable of agglutinating these cells.

The reagent was then found to contain antibodies capable of agglutinating cells of but two of the nineteen Dalles mice tested, and with the cells of these two the reaction was weak. The reagent agglutinated cells of twenty-one of twenty-five Bingen mice tested and of fourteen of seventeen Hood River mice tested (Table I). The antigenic component which reacted with reagent III is designated BH_1.

The tests indicate (Table I) that BH_1 is more widely distributed than is BH_2. BH_1 is present in many individuals which lack BH_2, but the converse never occurs, which seems to indicate some relationship between the two (see Discussion).

As far as the tests indicate, BH_2 is not present in any Dalles cells, whereas BH_1 is present in some of these cells. Evidence for this point is derived from two sources: by inference, since the Dalles cells used in absorbing reagent I removed BH_1, leaving BH_2; from the observation that the cells of two Dalles individuals were agglutinated, though weakly, by reagent III.

Reagent IV. After the preliminary absorption, this reagent was absorbed with Bingen cells found to contain BH_2 by tests with reagent I; all of them subsequently tested with reagent III contained BH_1 as well. Three

TABLE I

Results of Testing Cells of Individual Mice in Each of the Three Stocks, Using the Seven Reagents

See page 5 for explanation of symbols. Blanks in any column indicate that cells of the corresponding individuals were not tested with the reagent designed at the head of that column.

	Reagents						
	I	II	III	IV	V	VI	VII
Antigens	BH_2	DH_2	BH_1	DH_1	BD_1	DH_3	BD_2
Mice							
Dalles							
1144	−	±	−	+++	+	++	±
1146	−	−	±	++	+	±	−
1183	−	−	−	±			
1394	−	+	−	+			
1395	−	−	−	++	++		−
1396	−	−	−	±	±	+	
1397	−	−	−	+	+	+	+
1399	−	±	−				
1441	−	±	−	++	±	+	−
1442	−	+	−	++	+	−	−
1443	−	+	−	++	+	+	−
1485	−	−	±	+	++	+	−
1486	−	+	−	++			
1487	−	−	−	+	+	+	
1488	−	+	−	++	++	++	±
1489	−	−	−	−	++	−	−
1490	−	±	−	+++	+++	+	
1506							±
1508							+
1509							−
7033	−	−	−	±	++	±	
58164	−	+	−	+++	+	++	±
Bingen							
868	−	−	±	±	+	−	±
1134	−	−	+	++	+	−	−
1136	−	−	+	+			
1309	++	−	±	−	++	−	
1310	+						
1312	−	−	+	+	+	−	+
1314	+						
1316	+	−					
1318		−	±	−	+	−	−
1319	−	−	+	−	+	−	−
1320	−	−	−	+	+	−	
1321	+	−	±	−			
1322	−	−	+	+	−	−	
1325	−	−	+	+	−	−	
1326	++	−		−			
1363	++	−					
1365	−	−	+	+	+	−	
1366	−	−	+	+	+	−	+
1367	−	−	+	+	+	−	+
1368	−	−	+	+	+	−	+
1369	−	−	+	+	+	−	
Bingen							
1370	−	−	+	−			
1373	−	−	−	−	++	−	
1374	−	−	−	−	+	−	−
1470	−	−	±	+	+	−	
1471	++	−	±	−			
1472	−	−	++	+	+	−	−
1475	−	−	+	+	+	−	−
1476	−	−	−	+			
1477	−	−	+	+	±	−	±
Hood River							
1153	?						−
1154	?						−
1157	−	−	±	+	−	−	−
1158	−	−	±	+	−	−	−
1184	−	−	+	+	−	−	−
1185	−	±	+	++	−	−	
1186	−	−	±	+	−	−	
1187	−	−	±	+			
1272	−	−	+	++	−	−	
1273	−	−	−	+++	−	−	
1275	+	−	±	+	+	±	−
1276	?	−					−
1277	+	+	±	+++	+	+	
1278	?						−
1279	+	+	+	+	+	+	−
1280	−	−	+	±	−	−	−
1401	−						−
1402	−	−	±	+	−	+	
1403	?	−	−				−
1404	?						−
1405	+						−
1406	−	+	+	++	−	±	
1407	+	−	++	+	+	−	
1431	?						−
1432	?						−
1433	?	+	−				−
1435	−						−
1436	−						−
1437	?						−
1438	?						−
7619	−						−
7628	?						−
7898	±	−					

absorptions removed all antibodies capable of agglutinating the absorbing cells.

The reagent agglutinated cells of seventeen of twenty-six members of the Bingen stock tested, of all fifteen Hood River mice tested, and of seventeen of eighteen Dalles mice tested. This antigenic component is designated DH_1.

DH_1 is more widely distributed than is DH_2 (Table I); it is present in most members of the Bingen stock, all of which lack DH_2, and in a larger percentage of the members of the other two stocks than is DH_2. No individuals were found to possess DH_2 but not DH_1, although the converse occurred in many instances. This fact suggests a relationship between the two components which parallels the relationship between BH_1 and BH_2.

That the DH components are separate from the BH components is demonstrated by the facts (1) that no Dalles cells contained BH_2, whereas most of them contained DH_2, (2) that cells of but two Dalles mice tested contained BH_1 whereas cells of all but one of them contained DH_1, (3) that in tests of the other two stocks there seemed to be no regular relationship between the presence or absence of either of the DH components and the presence or absence of either of the BH components.

There is some evidence of cumulative action of DH_1 and DH_2. In many instances (Table I) macroscopic agglutination occurred with cells which contained both, although there were exceptions. Conversely, in most instances in which cells contained DH_1 but not DH_2 microscopic agglutination alone resulted; again, there were exceptions.

Reagent V. After preliminary absorption, this reagent was absorbed with Hood River cells which contained BH_1 and DH_1 but lacked BH_2 and DH_2. Two successive absorptions removed all antibodies capable of agglutinating these cells.

The reagent retained antibodies capable of agglutinating the cells of four of fourteen Hood River individuals tested, of all fifteen of the Dalles mice tested, and of eighteen of twenty Bingen mice tested (Table I). Apparently, the reaction was not due, primarily at least, to the presence of antibodies for BH_2 and DH_2. Cells of Hood River mice Nos. 1185 and 1406 were not agglutinated by reagent V, although tests with reagent II had shown that they contained DH_2. All Dalles cells had been found to lack BH_2, yet all were agglutinated by reagent V. All Bingen cells lacked DH_2 and most of them lacked BH_2, yet cells of all but two Bingen mice were agglutinated by reagent V. Finally, it is noteworthy that the cells of one Dalles individual (No. 1489) and of two Bingen individuals (Nos. 1373 and 1374) which contained none of the antigenic components previously identified were agglutinated, two of them strongly, by reagent V.

Evidently, therefore, reagent V contained antibodies against a component not previously isolated. This component is designated BD_1. Presumably, antibodies for BD_1 had been removed by all previous absorptions. This supposition is supported by the fact that the cells of all Dalles and Bingen individuals contributing to previous absorptions and extant for testing with reagent V were agglutinated by the latter.

Reagent VI. After preliminary absorption, this reagent was absorbed with Bingen cells which contained BH_1 and DH_1, but lacked BH_2 and DH_2. Three successive absorptions removed all antibodies capable of agglutinating these cells.

Reagent VI retained no antibodies capable of agglutinating cells of any of the Bingen mice tested. It agglutinated the cells of twelve of fourteen Dalles mice tested, and of five of fourteen Hood River mice tested (Table I). Reagent VI resembled reagent II in that all agglutinins for Bingen cells were removed; there seemed to be no correlation, however, between the reactions of the two reagents with cells of the other two stocks. Thus, the reaction produced by reagent VI was not dependent upon DH_2. Further evidence that agglutination could not have been due to DH_2 in some instances and BH_2 in others is afforded by the fact that the cells of one Hood River mouse and of six Dalles mice which contained neither component were agglutinated by reagent VI. Cells of Dalles No. 1442 and of Hood River No. 1185 possessed DH_2, but were not agglutinated by reagent VI. Similarly, cells of Bingen No. 1309 and of Hood River No. 1407 possessed BH_2, but were not agglutinated by reagent VI.

That the reaction of reagent VI could not have depended upon residual DH_1 was shown by the fact that cells of many of the Hood River mice which possessed DH_1 were not agglutinated by this reagent. Similarly, reaction did not depend upon residual BH_1, since many of the Dalles cells agglutinated by reagent VI lacked BH_1, and cells of several Hood River mice possessing BH_1 were not agglutinated by reagent VI. All but two of the individuals contributing cells for the absorption of reagent VI possessed component BD_1, hence antibodies for the latter must have been removed. Tests with reagent VI seemed to reveal no correlation with the results of tests with reagent V.

It seems evident, therefore, that the reaction of reagent VI depends upon an antigenic component not isolated in previous absorptions; this component is designated DH_3. As noted previously, no cells contained DH_2 which did not also contain DH_1. Similarly, no cells contained DH_3 which did not also contain DH_1, although many contained the latter without the former. These facts seem to indicate association between the three components which have been designated with "DH." Some cells contain DH_1 alone; some contain DH_1 and DH_2; some contain DH_1 and DH_3; some contain all three.

Reagent VII. After preliminary absorption, this reagent was absorbed with cells from the Hood River stock in an attempt to remove all antibodies capable of agglutinating cells of this stock. Three successive absorptions removed all agglutinins for cells of extant mice in the stock.

Reagent VII contained antibodies which agglutinated cells of six of fourteen Dalles mice tested, and of six of twelve Bingen mice tested (Table I). It was hoped that the absorption would remove antibodies for all antigenic components previously identified. Attainment of this goal was rendered difficult by the small number of animals remaining available, but one at least of the mice contributing to the absorption of this reagent (No. 1279) had been shown to possess all six of the components previously isolated. Although the tests are few and, consequently, not as conclusive as might be desired, it does not seem possible to ascribe the agglutination produced by reagent VII to any of the components identified previously; the antigenic component involved is therefore designated BD_2.

It will be noted that some cells contained BD_1 but not BD_2; the latter was never shown to occur without the former. Thus, it seems that there exists the same relationship between BD_1 and BD_2 as exists between BH_1 and BH_2, DH_1 and DH_2, or DH_1 and DH_3.

DISCUSSION

By use of parts of antiserum absorbed in seven different ways, seven different antigenic factors or components have been isolated. There is no evidence that the entire number present in erythrocytes of these stocks has been discovered or that the components which have been isolated have been resolved into their smallest constituent units.

The practical necessity of employing pooled cells for absorption renders difficult if not impossible the isolation of ultimate antigenic units dependent upon single genes after the manner of the human haemagglutinogens, the antigenic factors isolated in doves and pigeons by Irwin and coworkers (1936; 1939; 1940; 1943), or those identified in cattle by Ferguson (1941) and Ferguson, Stormont, and Irwin (1942). Gorer (1936*b*; 1937) mitigated the disadvantage imposed by use of pooled cells by employing stocks of mice which had been inbred, brother to sister, for over twenty generations. In consequence his stocks approached homozygosity, although the transplantation experiments of Loeb (1945: Chap. 9) suggest that prolonged inbreeding of mice does not result in as close an approach to identity of "individuality differential" as had sometimes been supposed. Nevertheless, the stocks employed by Gorer must have been much more uniform in antigenic structure than were the stocks used in the present investigation. Even approximate homozygosity can hardly be expected in any investigation upon mammals obtained from natural habitats or upon the immediate descendants of such animals.

Despite the handicaps imposed by the nature of the material, however, antigenic components in these stocks of deer-mice have been discovered, and some progress has been made toward analysis of their inter-relationships. The seven components occur in three groups or complexes. The "BH complex" (BH_1 and BH_2) comprises antigenic factors possessed in common by many Bingen and Hood River mice, but possessed by few, in the case of BH_1 or no, in the case of BH_2, Dalles mice. Similarly, the "DH complex" (DH_1, DH_2, DH_3) consists of components possessed in common by many Dalles and Hood River mice; DH_1 is also present in cells of a considerable proportion of Bingen mice, whereas DH_2 and DH_3 are absent from the latter. In like manner, the "BD complex" (BD_1 and BD_2) is composed of antigens shared by cells of many Bingen and Dalles mice; BD_1 is also present in cells of about one-fourth of the Hood River mice, and BD_2 is lacking in the latter stock.

A schematic representation of these complexes and components is given in Figure 1. The entire square represents the total antigen content of a red blood cell. The greater proportion of this content, represented by the lower two-thirds of the square, is shared in common by all mice of the species *Peromyscus maniculatus*; in this investigation the corresponding antibodies were removed by the preliminary absorption with the cells of the Oyster Bay stock. The three squares into which the upper third of the diagram is divided represent the three "complexes" described above; the subdivision of each into its constituent antigens is indicated. The cells of no one mouse contained all components included in the diagram.

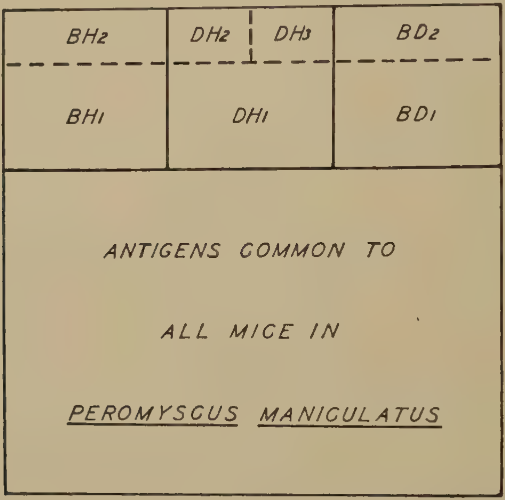


FIG. 1. Schematic representation of the relationships of the antigenic factors identified in the present study.

That these three "complexes" are real entities is suggested by a number of facts. In each one the antigens designated with the subscripts "2" and "3" were not found to occur in cells which did not have also the corresponding antigen designated with the subscript "1." The converse occurred frequently, however, as noted previously. The basic relationship of the component designated as "1" is also suggested by the results of the absorption of reagents V and VI. In each instance the attempt was made to remove agglutinins for BH_1 and DH_1, leaving antibodies for BH_2 and DH_2. The results seemed to indicate that antibodies for the latter were also removed, despite the fact that previous tests had failed to demonstrate the presence of BH_2 and DH_2 in cells of the kind used for absorption. Apparently, massive absorption with cells containing BH_1 (or DH_1) will remove not only antibodies for the latter but also antibodies for BH_2 (or DH_2, respectively). The amount of BH_1 in Dalles cells used to absorb reagent I was apparently too small to remove all antibodies for BH_2.

The nature of the relationship between the several components comprising one "complex" cannot be determined from the present investigation. Similarity of chemical structure would seem to offer the most reasonable explanation. There is much evidence that antibodies react not only with the antigen which elicited their formation but also with antigens of similar chemical structure (cf. Landsteiner, 1945: 54). According to this view DH_1 would be sufficiently similar to DH_2 so that antibodies for the latter could be removed by massive absorption with the former. Somewhat comparable situations are apparently presented by absorption of agglutinin α_1 from human sera of groups O or B by cells of group A which lack agglutinogen A_1 (Landsteiner and Witt, 1926), and by absorption of agglutinins for the factor N by human cells which are negative in the agglutination test for that factor (Landsteiner and Levine, 1928).

Of interest in this connection is the fact that although the antiserum was produced by inoculation of a rabbit with cells from Dalles mice, agglutinins were formed which were not completely absorbed by the cells of Dalles mice. As remarked by Boyd and Warshaver (1945): "It is certainly seldom that the observation is made that an antigen will not absorb out all the antibodies which it causes an animal to produce." Yet, in the present instance, the antiserum contained antibodies for a component (BH_2) seemingly not in the homologous (Dalles) cells, as well as for a component (BH_1) which was of apparently rare occurrence in them. Boyd and Warshaver (1945) reported four cases in which injection into rabbits of group B human erythrocytes resulted in the production of anti-A as well as of anti-B antibodies. They regarded the result as confirming the idea that the A and B antigens may be chemically similar. Their observations and mine accord well with the conception of Landsteiner (1945: 114 and 270–71), based upon experiments with immune sera for azoproteins and for ovalbumin (Landsteiner and van der Scheer, 1940), that the formation of several antibodies may be elicited in response to a single determinant group, that antibodies so formed "vary to some extent around a main pattern, and that what is ordinarily spoken of as an antibody is generally a mixture of specifically different components." Similar views have been expressed by other writers.

Since most of the mice which furnished cells for immunization of the rabbit did not survive for subsequent testing, there is some slight possibility that some of them possessed antigenic components not found in the Dalles mice which were tested. In view of the results of others this seems a less probable explanation for the occurrence of antibodies not completely absorbed by homologous cells than does the hypothesis given in the preceding paragraph.

Great individual variation occurred in the reaction of cells with the seven reagents (Table I). The cells of two individuals (Hood River Nos. 1277 and 1279) were agglutinated by six of the reagents. At the other extreme were individuals the cells of which were agglutinated by but one of the seven reagents. A variety of intermediate conditions existed. It is of interest that the two extremes were within a single stock (Hood River). This observation is in accord with the findings concerning agglutinogens in other animals (e.g., A and B in man) that differences between individuals within one race may be as great as differences between individuals belonging to different races.

Although the material of the present investigation does not lend itself to genetic analysis, some evidences of inheritance of antigenic components were found. Hood River mice Nos. 1277 and 1279, the only individuals that possessed six of the seven components, were sibs, another sibling being No. 1275, which possessed five components. At the other extreme was a litter mate, No. 1280, which possessed but two components. Nos. 1406 and 1407, offspring of the same parents as these others, possessed four components, variously assorted, the differences between the two individuals (Table I) suggesting the heterozygosity of the parents. Four of the five Hood River mice found to possess DH_3 were in this one family, as were all the Hood River mice that possessed BD_1. Somewhat similar family groupings occurred in the other stocks, but the present findings can serve merely to suggest interesting possibilities if future serological investigations on *Peromyscus* are planned to make genetic analysis possible.

Figure 2 presents "profiles" of the three stocks in terms of the percentages of individuals in each stock which possessed each of the seven agglutinogens. The percentages are of relative and approximate significance merely, since, as mentioned previously, there is no assurance that each stock presents a truly random sample of the population from which it was derived. Some essential points of difference between the stocks are emphasized by the "profiles." Each stock is characterized by possession in many individuals of two antigenic complexes, each shared largely with one of the other stocks and slightly, if at all, with the third one. Four of the components seem to be completely lacking from one or another of the stocks. Only one of the components (DH_1) has really considerable representation in all three stocks, although a second (BD_1) is present in a high percentage of the members of two stocks and in about 28 per cent of the third.

Thus, it is not possible to state that one stock is characterized by the presence in the cells of all individuals of an agglutinogen which is absent from the cells of all individuals of other stocks. A negative characterization is possible due to the fact that each stock may be said to be characterized by the absence of one or more agglutinogens present in the others. But on the whole the agglutinogens are shared by the stocks, each stock differing from the others in the proportion of the population possessing each of the antigenic factors. Characterization of stocks becomes statistical, therefore, as in the characterization of human racial groups by means of agglutinogens A and B.

In this respect the present findings agree with those of other similar investigations. Thus, Gorer (1936*b*; 1937), investigating inbred strains of agouti, albino, and black mice, isolated four antigenic factors. One of these was lacking from cells of the black strain; otherwise the four were present in all three strains, but the latter differed in the relative strength of the reaction by which the cells responded to antibodies for the respective factors. Landsteiner (1945: 117) investigated two species of ducks of such close relationship that hybrids were completely fertile. Using rabbit immune sera he was able "to demonstrate an agglutinable factor which occurs more frequently in one species but does not permit the differentiation of every individual." Owen, Stormont, and Irwin (1944) studied the differences in frequency between Guernsey and Holstein-Friesian cattle of thirty cellular

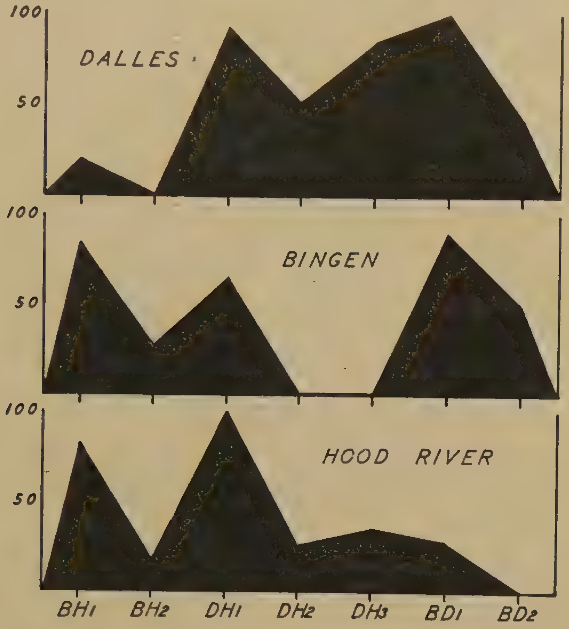


FIG. 2. Relative numbers of individuals in each of the three stocks that possessed each of the seven antigenic components. The ordinates are plotted in per cent of population exhibiting the components.

antigens. They reported that "no single antigen by itself distinguishes these two breeds. Rather, the breed differences in blood type are the result of quantitative differences in the frequencies of most of the antigens."

If the animals investigated are less closely related than were the ones used in the present investigation or those used in the studies cited immediately above, the overlapping in agglutinable substances is less marked and in consequence differentiation becomes more clear cut (cf. Landsteiner 1945: 78). Thus, Moody (1941) using absorbed antisera to identify individuals in two distinct species of *Peromyscus* found differentiation very definite. Apparently, most of the antigenic components of one species were not shared with the other. A few individuals gave evidence, however, of possessing a component shared with the other species, precisely in the manner in which the three closely related stocks of the present investigation possessed agglutinogens in common.

SUMMARY AND CONCLUSIONS

Antigenic constitution of the red blood cells of three stocks of deer-mice from the Columbia River Valley was investigated with absorbed reagents prepared from immune serum for the cells of one of the stocks. The three stocks were very similar in morphology, and this similarity extended to antigenic structure. Most of the cell antigens were shared by all the mice; when antibodies for these antigens had been removed from the antiserum, seven antigenic components of more limited distribution were identified (Table I and Fig. 1). Although four of these components were lacking from the cells of one or another of the stocks, for the most part the components were shared by the stocks, but possessed by varying proportions of the population of each.

Accordingly, characterization of the stocks must be mainly quantitative, rather than qualitative. The stocks resemble mosaics in which each of the constituent units varies independently in size, so that one pattern is never the duplicate of another. This mosaic pattern in distribution of antigens resembles the varying mosaics of morphological characters reported by Dice (1937: 17–20; 1939*a*; 1939*b*) to characterize local and geographical races. Thus, the average femur length of one local race may differ significantly from that of a neighboring local race. This difference may or may not be correlated with differences in other characters (e.g., length of mandible, length of ear, color of pelage). In like manner possession of an antigen in 90 per cent of the population may characterize one local race, possession of it in 10 per cent of the population may characterize another local race. This difference may or may not be correlated with differences in distribution of other antigens or with differences in morphological characters. Accordingly, the usefulness of cell antigens in studies of distribution and speciation will depend upon the nature of the problem at hand. The advantages and limitations are much the same as those which accompany attempts to apply to anthropological problems data concerning human blood groups and types.

Since all evidence points to genetic determination of antigens, it would seem that cell antigens might offer a valuable means by which to trace gene interchange between neighboring populations of mammals as well as the fluctuating distribution of genes throughout taxonomic and distributional groupings of higher order. A necessary preliminary to studies of this kind would be the isolation of antigenic units and determination of their genetic basis. From the results of investigations on other animals it would seem that isolation of antigens dependent upon single genes should be possible. For reasons discussed previously, pooling of blood from different individuals should be avoided if possible. If use of pooled cells proved a practical necessity, the raising of highly inbred strains as practiced by Gorer (1936*b*) would be essential. There would thus be made available requisite quantities of cells of uniform antigenic constitution for use in production of antisera and subsequent absorption of the latter. Sufficient quantities of uniform reagents for the testing of cells of wild populations could thus be secured.

The following observations are recorded and discussed: (1) individual differences in number of antigenic factors possessed were in some cases as great between members of the same stock as they were between members of different stocks; (2) under some circumstances absorption with cells containing one antigenic component removed antibodies for another one; (3) absorption with cells of the kind with which the rabbit producing the antiserum had been immunized did not remove all antibodies from the antiserum.

Two of the stocks (Dalles and Hood River) originated from the south side of the Columbia River; one (Bingen) came from the north side. A point of interest was whether or not the river in this region formed a barrier to the dispersal of deer-mice. The stocks were so similar in antigenic constitution that no evidence of separation by a barrier was afforded by the present investigation.

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VARIATION IN THE PYGMY MOUSE (*BAIOMYS TAYLORI*) FROM TEXAS AND ARIZONA

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INTRODUCTION

LABORATORY studies of variability in natural populations of mammals have been necessarily limited to the genus *Peromyscus* because most other kinds of small mammals tested have not bred satisfactorily in captivity. The discovery that the pygmy mouse (*Baiomys taylori*) is a satisfactory laboratory animal (Blair, 1941) has made possible the investigation of the variability of *Baiomys* populations by raising laboratory stocks of known age. The present report deals with variation in a stock of *Baiomys taylori subater* (Bailey) from Texas and in a stock of *Baiomys taylori ater* Blossom and Burt (1942) from Arizona.

METHODS

Field-caught stocks of pygmy mice were shipped alive to the Laboratory of Vertebrate Biology at the University of Michigan, where they became the parents of the laboratory-bred populations. The mice were raised under the standard conditions developed for *Peromyscus* (Dice, 1929). They were killed at an age of approximately four months and then were prepared as flat skins with partial skeletons. Body measurements of the freshly killed mice were made by the senior author. Standard skeletal measurements (Dice, 1932) were made by several assistants. Color measurements were made by the same assistants, using the Ives tint photometer. Because of the small size of these mice, color readings were made only on the dorsal stripe. The statistical calculations were made by D. M. Clarke, William Jalosky, and the senior author. All expenses of this project except the collecting of the stock of *Baiomys taylori ater* and the preparation of the manuscript were supported by the Laboratory of Vertebrate Biology.

STOCKS OF MICE

The origin of the two stocks studied is as follows:

Baiomys taylori subater—La Belle. This stock descended from nine field-caught mice that were trapped by W. F. Blair on January 25 and 26, 1940, about seven miles southeast of La Belle, Jefferson County, Texas. The mice were trapped in a small area of dense tall grass (*Andropogon* sp.) surrounding a pond on a low moist coastal prairie.

Baiomys taylori ater—Hereford. This stock originated from four males and two females that were trapped by P. M. Blossom on March 26 and 27, 1941, about nine miles west of Hereford, Cochise County, Arizona. The mice were trapped along a roadside where tall grass and weeds formed a rather heavy ground cover. The mice were all obtained from a narrow strip of grass and weeds about 20 by 1300 feet, adjoining a fenced, pastured field. The elevation was 4800 feet. Field-killed specimens from the same general locality were used as the basis for describing the geographic race *ater* (Blossom and Burt, 1942).

SOIL COLORS

The two stocks come from regions of contrasting trends in soil color. The *subater* mice were living on the dark brown soils of the eastern Texas coastal prairie. The surface soil in that region is relatively low in the reflectance of red, green, and blue-violet (Table I), hence is dark in shade. The soil on which the *ater* mice were living is reddish in tone and is relatively darker than the other desert soils of the region. A sample of surface soil from six miles west of Hereford, however, is almost twice as high in tint photometer readings (i.e., paler) with all color screens than is the soil from the Texas coastal prairie. Therefore, the *ater* mice were living on soils that were paler than were the soils on which the *subater* mice lived.

TABLE I

COLOR OF SURFACE SOIL ON THE TEXAS COASTAL PRAIRIE, FIVE MILES SOUTHEAST OF LA BELLE, AND ON THE ARIZONA DESERT, SIX MILES WEST OF HEREFORD

Means of Ten Tint Photometer Readings.

Station	Red	Green	Blue-Violet
La Belle, Texas	13.8	10.4	7.7
Hereford, Arizona	25.6	18.6	14.7

TABLE II

MEASUREMENTS OF *Baiomys taylori subater* DESCENDED FROM A TEXAS POPULATION AND OF *Baiomys taylori ater* DESCENDED FROM AN ARIZONA POPULATION

Measurements and standard errors, in millimeters.

Stocks	Specimens	Mean Age in Weeks	No. of Parents	Body Length	Tail Length	Foot Length	Ear Length
B. t. subater ♀	25	27	9	66.80 ± .57	42.60 ± .73	13.272 ± .135	10.176 ± .150
B. t. subater ♂	30	27	9	64.60 ± .34	40.31 ± .58	13.333 ± .089	10.103 ± .102
B. t. ater ♀	30	25	6	70.23 ± .78	45.68 ± .50	14.333 ± .072	10.550 ± .077
B. t. ater ♂	23	26	6	67.96 ± .42	44.89 ± .53	14.222 ± .077	10.400 ± .107

	Left Femur	Left Mandible	Condylo-Premaxilla	Condylo-Zygoma	Bullar Width
B. t. subater ♀	11.163 ± .090	11.148 ± .082	16.942 ± .118	12.896 ± .080	8.270 ± .045
B. t. subater ♂	10.650 ± .062	10.800 ± .043	16.525 ± .074	12.592 ± .058	8.200 ± .035
B. t. ater ♀	11.664 ± .105	11.138 ± .062	17.186 ± .082	13.138 ± .057	8.179 ± .039
B. t. ater ♂	11.232 ± .084	10.930 ± .071	16.848 ± .069	12.876 ± .047	8.123 ± .036

INTERPOPULATION FERTILITY

Two *subater* females were mated to *ater* males. One of these females bore three litters consisting of seven hybrid individuals. The other female bore two litters containing a total of five hybrid offspring. The two strains crossed readily, and the resultant litters were of normal size. One pair of F_1 hybrids was mated and produced a litter of three and a litter of one F_2 hybrids. Therefore, the two populations from about a thousand miles apart are interfertile to the extent of being able to produce fertile hybrids in interpopulation crosses.

VARIATION DUE TO SEX

Female specimens of *Baiomys,* like female specimens of *Peromyscus,* average generally larger than do males from the same populations. The *subater* females average significantly larger than do the males in body length, femur length, length of mandible, condylo-premaxillary length of skull, and condylo-zygomatic distance (Table II). There is no significant difference between the sexes in tail length, foot length, length of ear from notch, or bullar width of the skull. The *ater* females average significantly larger than do the males in femur length, condylo-premaxillary length of skull, and condylo-zygomatic distance. The females probably are also larger than the males in body length (P between 0.05 and 0.01). There is no significant difference between the sexes of *ater* in tail length, foot length, ear length, length of mandible, or bullar width of the skull.

As there is no significant difference between males and females of either stock in pelage color, the sexes have been pooled for comparisons between the stocks.

GEOGRAPHIC VARIATION IN DIMENSIONS OF BODY AND SKELETON

The specimens of *ater* from Arizona average significantly larger in certain body and skeletal measurements than do those of *subater* from Texas (Table II). The *ater* males are larger than the *subater* males in body length, tail length, foot length, length of femur, and condylo-zygomatic distance. The males of the two stocks do not significantly differ in length of mandible, condylo-premaxillary length of skull, or bullar width of skull.

The female specimens of *ater* average significantly larger than the *subater* females in body length, tail length, foot length, and femur length. There is no significant difference between the females of the two stocks in length of mandible, condylo-premaxillary length of skull, condylo-zygomatic distance, or bullar width of skull.

VARIATION IN PELAGE COLOR

The *ater* mice from Arizona average very significantly higher in tint photometer readings (i.e., paler in pelage color) than do the *subater* mice from the Texas coastal prairie (Table III). The two stocks differ most in

TABLE III

Pelage Color of *Baiomys taylori subater* from Texas and of *Baiomys taylori ater* from Arizona

Mean tint photometer readings, with standard errors. Males and females combined.

Stocks	Red	Green	Blue-Violet
B. t. subater	4.11 ± .08	2.89 ± .07	2.26 ± .07
B. t. ater	6.37 ± .12	4.48 ± .10	3.41 ± .10
Differences of *ater* from *subater*	+ 2.26 ± .14	+ 1.59 ± .12	+ 1.15 ± .12

the readings for reflected red, for the difference is 2.26 ± 0.14 tint photometer units (Table III). They differ least in the readings for reflected blue-violet with a difference of 1.15 ± 0.12 units.

Pygmy mice (*Baiomys taylori*), as represented by these two stocks, are less variable locally in color than are many populations of the genus *Peromyscus*. The *ater* stock, which is the more variable of the two in pelage color, has a standard deviation for reflected red of only 0.85 ± 0.08 tint photometer units. By comparison seventeen of eighteen stocks of *Peromyscus maniculatus* measured by Dice (1941) from the region of the Nebraska sandhills have significantly higher standard deviations for reflected red than does the stock of *ater*. The *subater* is less variable than is *ater*, with a standard deviation for reflected red of only 0.61 ± 0.06.

DISCUSSION

The pygmy mouse (*Baiomys taylori*) shows the same trend toward correlation of pelage color with soil color as do mice of the genus *Peromyscus*. In the case of the two stocks studied the mice from the paler soil were slightly but very significantly paler than those from the darkest soil (Table III). The tendency for pygmy mice to be paler on pale soils than they are on dark soils presumably is attributable to natural selection.

It is noteworthy that the tint photometer readings of the laboratory-reared mice show *ater* to be the paler of the two subspecies, although *ater* was described by Blossom and Burt (1942) as the darkest race of the species *taylori*. It should be mentioned in this connection that the type series of *ater* was not statistically evaluated for color, and these mice were of various ages of field-caught animals that were prepared in the field as round skins. This discrepancy between the tint photometer readings of the laboratory-reared mice and the description of *ater* suggest that accurate comparisons of the pelage color of different populations of mammals can be made only by colorimetric methods, using animals of known age and pelage.

The differences between the two populations are of the same sort as have been reported between geographically distant populations of *Peromyscus*. The *ater* mice are significantly larger than the *subater* mice of corresponding sex in body length, tail length, foot length, and length of femur. The *ater* males are also larger than *subater* males in condylo-zygomatic distance.

The pygmy mice show little local or geographic variability in pelage color. The standard deviation for reflected red is only 0.61 ± 0.06 tint photometer units in the *subater* mice and 0.85 ± 0.08 units in the *ater* mice. The color differences between the two stocks, although highly significant statistically, are of low magnitude.

In tint photometer readings for reflected red the *ater* are only 2.26 ± 0.14 units higher (i.e., paler) than are the *subater*. With the green and blue-violet screens the differences are even less, although highly significant statistically (Table III). As indicated by these stocks the pygmy mouse (*Baiomys taylori*) appears to have a low range of genetic variability in pelage color.

SUMMARY

Stocks of pygmy mice, *Baiomys taylori subater* from seven miles southeast of La Belle, Jefferson County, Texas, and *Baiomys taylori ater* from nine miles east of Hereford, Cochise County, Arizona, were bred in the laboratory and raised to an age of approximately four months to determine the variability in these mice.

Cross matings between the two stocks indicated their interfertility. Both an F_1 and F_2 generation were obtained.

The females in both stocks averaged significantly larger than the males in several body and skeletal measurements. The *subater* females exceeded the males in body length, femur length, length of mandible, condylo-premaxillary length of skull, and condylo-zygomatic distance. The *ater* females exceeded the males in femur length, condylo-premaxillary length of skull, condylo-zygomatic distance, and probably also in body length. There was no significant difference between sexes in pelage color.

The *ater* mice were significantly larger than the *subater* in several measurements. The *ater* males exceeded *subater* males in body length, tail length, foot length, length of femur, and condylo-zygomatic distance. The *ater* females exceeded the *subater* females in body length, tail length, foot length, and femur length.

The *ater* mice from relatively pale soil were slightly but very significantly paler than the *subater* mice from relatively dark soil. The differences in tint photometer readings for the two stocks were 2.26 ± 0.14 units for red, 1.59 ± 0.12 for green, and 1.15 ± 0.12 for blue-violet.

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